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THE FATTY ACIDS OF HUMAN DUODENAL BILE, THEIR QUANTITATIVE SEPARATION, ESTIMATION AND THE EFFECT OF FOODSTUFFS ON THEIR SECRETION

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The present communication describes a method for the quantitative separation and estimation of the fatty acids of bile lipids; the bromine numbers of the total fatty acids of human duodenal bile; the bromine numbers of total fatty acids of blood samples withdrawn during the periods of collection of duodenal bile specimens; and the effects of intraduodenal administration of pure foodstuffs on the lipids and phosphorus compounds of human duodenal bile.

Studies on the lipids and phosphorus compounds of bile derived from the human gall bladder and from fistulae resulting from operations on human subjects have been made by various investigators, including the present authors. The results of all these investigations have been correlated and published (1). Observations on duodenal bile have been greatly hampered by the paucity of suitable analytical methods. The development of such methods and their application to the study of bile derived by means of a sound from the duodenum of normal men has made possible the present investigation.

For purposes of this study the duodenal tube was swallowed by three normal young men, in the morning and 14 to 16 hours after the last ingestion of food. The tip of the tube was allowed to pass into the second portion of the duodenum where its position was verified by means of the x-ray fluoroscope. Then 100 cc. of 1, a warm aqueous mixture of 50 cc. of pure cottonseed oil; 2, a solution of 50 grams of fat-free beef peptone,[1] or 3, a

[1] The peptone was prepared by the Arlington Chemical Company, Yonkers, N. Y., to whom we express our thanks.

solution of 50 grams of pure dextrose were slowly run into the duodenum through the tube. For a period just prior to the introduction of a foodstuff a specimen of the duodenal contents was collected, which represented the conditions of the fasting state. After administration of the various foodstuffs, collection of duodenal contents was begun. Separation of specimens for analysis was commenced when the light yellow bile-colored duodenal contents changed to a darker color described as some shade of brown. Such specimens were collected over periods usually not exceeding 30 minutes. A series of fractional collections was made until one of much lighter color was obtained which indicated that stimulation was diminishing or had ceased. This procedure for collection of specimens was followed because numerous previous observations, some of which have been published (2), have established that stimulation of both the flow of bile and pancreatic enzymes is evidenced by the presence of dark colored bile in the duodenal contents. All specimens of duodenal bile were collected directly into alcohol in graduated cylinders.

Samples of blood were withdrawn from the median basilic vein during the various periods in which specimens of duodenal bile were collected. The total fatty acids of the blood were separated as described by Bloor (3). The total fatty acids of bile were extracted and purified as described for the oxidation method which is reported later in this communication. They were finally dried in a vacuum desiccator to constant weight and the bromine number determined.

The fats, soaps and free fatty acids were separated, their fatty acid fractions and also the total fatty acids of the duodenal bile specimens were quantitated by methods presently to be described in the body of the text. Phosphorus from the organic and inorganic sources in duodenal bile was quantitated by methods previously reported (4). Bromine numbers of the total fatty acids of both bile and blood were estimated by an unpublished method, which permits distinction to be made between addition and substitution. The method gives reliable results for as small an amount of fatty acids as can be weighed accurately.

The method devised for the study of bile lipids required a large amount of experimental work in which various fat solvents and numerous technical procedures were studied before a satisfactory method for separating and estimating the esterified fatty acids was evolved. During the course of this work observation established that fat solvents will readily dissolve the biliary fatty acid compounds if the physical state of the bile preparation will permit direct contact of the fats with the solvent. Investigation demonstrated further that this could be accomplished by various procedures one of the most satisfactory of which was the extraction of the fats from bile dried on strips of filter paper. One or 2 cc. of bile will dry at room temperature in less than 30 minutes and but little manipulation is

required to extract the organically combined fatty acids. However, in attempting to separate these compounds from soaps it was found that benzene, chloroform and other solvents in which soaps are ordinarily difficultly soluble would partially extract them from bile when the latter is dried on strips of filter paper. Further observation established that under such conditions soaps were practically insoluble in cold carbon tetrachloride. In a period of 12 hours no solution occurs; in a period of 40 hours amounts varying from a trace to not more than 4 per cent will dissolve. The fats and other organically combined fatty acids of bile dried on strips of filter paper are extracted by cold carbon tetrachloride very readily as a rule; usually extractions of a strip over three fifteen minute periods will suffice. However, probably due to mechanical interference, it is necessary occasionally to carry on the process for five or six hours to procure complete extraction. To insure uniformly complete extraction as well as for convenience, an initial extraction period of 16 hours was chosen; this permits the specimen to stand overnight. The method consists essentially of drying bile on filter paper, extracting with cold carbon tetrachloride, saponifying the extract, purifying the fatty acids thus obtained and estimating their amount by either a neutralization or an oxidation method. The neutralization method consists in titrating with an alcoholic solution of sodium hydroxide, using alphanaphtholphthalein as the indicator. The oxidation method is that described by Bloor (5). The reagents used for the first method are as follows: redistilled 95 per cent ethyl alcohol; redistilled ethyl ether; the petroleum ether fraction which distills below 55° to 60°C.; 0.8 per cent solution of alphanaphtholphthalein in 60 per cent (by volume) redistilled ethyl alcohol; a saturated aqueous solution of sodium hydroxide; a mixture of equal volumes of H_2O and C.P. HCl; the "Thiophene-free" C.P. commercial preparation of benzene; C.P. carbon tetrachloride. An approximately N/10 solution of sulphuric acid is prepared and its normality accurately determined. An approximately N/10 alcoholic solution of sodium hydroxide is also prepared. For the oxidation procedure a sulphuric acid solution of silver dichromate and solutions of potassium thiosulphate, potassium iodide and of starch are prepared as directed by Bloor (5).

Procedure for partitioning and estimating fatty acids of combined fats and free fatty acids. Twenty cubic centimeters of bile are pipetted into a 100 cc. volumetric flask. Then about 70 cc. of equal parts of the redistilled alcohol and ether are added, the contents thoroughly mixed and the volume completed to 100 cc. by more alcohol-ether. After standing a minimum of five minutes, the alcohol-ether extract is filtered into a narrow necked glass stoppered bottle and tightly stoppered. The volume of the precipitate is negligible. This filtrate is used both for estimation of total fatty acids and of fats. Twenty-five cubic centimeters of the filtrate (equiva-

lent to 5 cc. of bile) are used for the determination. This amount is pipetted into a 100 cc. pyrex beaker and evaporated to dryness on the steam bath. The residue is dissolved in exactly 5 cc. of water, solution occurring rapidly and completely. Of this 2 cc. portions are spread on strips of filter paper about 5 × 30 cm., suspended in mid air and dried at room temperature. The dried strip is rolled and thrust into a pyrex Erlenmeyer flask of 125 cc. capacity. Sufficient carbon tetrachloride is added to cover the filter paper, the flask covered with a watch glass and the whole allowed to stand at room temperature 16 hours. The carbon tetrachloride is next decanted into a 150 cc. beaker from which it is quantitatively transferred in successive 25 cc. portions to a 50 cc. Erlenmeyer flask in which evaporation is being carried out on the electric hot plate as follows: The small Erlenmeyer flask is stoppered with a cork through which extends a bent glass tube of about 3 mm. diameter. The distal end of the tube dips into a kidney-shaped basin partially filled with mercury kept cool by an ice cube. The carbon tetrachloride fumes condense in the mercury and can be separated from the water resulting from liquefaction of the ice in a separatory funnel, redistilled and again used. The roll of filter paper is then extracted twice more over successive periods of two and one hours each. The collected extractions are evaporated nearly to dryness in the 50 cc. Erlenmeyer flask on the electric hot plate and the drying finally completed on the steam bath. When no more than traces of carbon tetrachloride remain 25 cc. redistilled 95 per cent alcohol and 0.5 cc. of the saturated aqueous solution of sodium hydroxide are added to the residue, the mixture boiled on the steam bath for 30 minutes and the alcohol evaporated. The residue is moistened with an amount of the aqueous HCl solution which represents not less than 0.25 cc. more of the acid solution than that amount necessary to neutralize the 0.5 cc. of NaOH solution originally added. Then enough H_2O is added to make the total amount of liquid equal to about 1.5 cc. The fatty acids are liberated by mixing the acid solution with the saponified residue by gentle rotation of the flask. Next the contents of the flask are heated on the steam bath for a few minutes, 20 cc. of redistilled petroleum ether added and actively boiled, then allowed to cool and decanted through a 7 cm. filter paper into a 125 cc. pyrex Erlenmeyer flask. This extraction is repeated six times. The combined petroleum ether extracts are evaporated to dryness on the steam bath. For the neutralization method the residue need not be entirely free from petroleum ether because the latter does not carry over HCl, as shown by absence of acidity and of $AgNO_3$ precipitate in blank control tests. At this stage the residue containing fatty acids and cholesterol is dissolved by the addition of exactly 10 cc. of redistilled alcohol and titration carried out as will be described later. On the other hand, if the fatty acids are to be oxidized the last traces of petroleum ether are removed by placing the

flask in the vacuum desiccator for about 15 minutes. To the residue are now added about 15 cc. of redistilled alcohol and 0.2 cc. of saturated NaOH solution and saponification again produced as previously described. The alcohol is evaporated and the residue completely dried by placing the flask in the vacuum desiccator for a few hours. To the dried residue are added 15 cc. of cold chloroform and the whole allowed to stand 15 minutes. The chloroform is poured through a small filter paper in order to catch any of the residue mechanically held by it. This process of extraction is repeated three times. The filter paper and the flask are now dried by placing in the vacuum desiccator until the chloroform has evaporated, a comparatively few minutes. The residue in the flask is dissolved by adding to it three portions of 5 cc. each of hot redistilled alcohol, which are poured through the same filter paper used in making the chloroform extract. The alcohol is now evaporated on the steam bath, the residue completely dried in the vacuum desiccator, acidified with about 1.5 cc. of acid solution and extracted with petroleum ether, as described before. The petroleum ether extract is decanted or filtered into a 250 cc. pyrex glass stoppered flask, the ether evaporated on the steam bath and the last traces removed by placing in the vacuum desiccator for about 30 minutes. The oxidation of the fatty acids is now carried out as described by Bloor (5).

The titration in the neutralization method is carried out as follows: First, the stock solution of sulphuric acid, whose normality has been predetermined, is diluted quantitatively with water to one-tenth of the original strength. The alcoholic sodium hydroxide solution is similarly diluted to $\frac{1}{10}$ strength with redistilled 95 per cent alcohol. The normality of the thus diluted H_2SO_4 solution is known and that of the diluted NaOH solution can now be determined by titration. This is accomplished by adding the diluted alcoholic NaOH solution from a microburette to 10 cc. of the aqueous diluted H_2SO_4 solution using 0.1 cc. of the 0.8 per cent alphanaphtholphthalein as the indicator. This procedure gives an approximately N/100 alcoholic NaOH solution of known concentration; and should be freshly prepared for each set of determinations. To the alcoholic solution of the fatty acids and cholesterol of the bile obtained as described previously is added 0.1 cc. of the 0.8 per cent alphanaphtholphthalein indicator and the acidity titrated with the N/100 alcoholic NaOH solution. The amount of NaOH solution used in the titration minus that necessary to neutralize 10 cc. of the redistilled alcohol used as the fatty acid solvent permits the calculation of the milligrams of fatty acid present; 0.354 cc. of N/100 NaOH is equivalent to 1 mgm. of oleic acid.

Free fatty acids in the bile are determined indirectly. Their amount represents the difference between the weights of fatty acids derived from both fats and free fatty acids and from fats alone. The fats of bile are isolated as follows: 25 cc. of the alcohol-ether extract are dried on the

steam bath. The resulting residue is dissolved in exactly 5 cc. of N/10 NaOH. This converts the free fatty acids to soaps. Two cubic centimeters of this alkaline solution are dried on strips of filter paper. The remainder of the procedure is that already described above for the estimation of the fatty acids of the combined fats and free fatty acids.

The determination of free fatty acids was considered necessary because, strange to say, the concentration of free fatty acids or of soap apparently does not depend on the small degrees of alkalinity or acidity found in bile.

Isolation of total fatty acids from bile. The procedures for isolating the total fatty acids from bile are outlined as follows: 10 cc. of the alcohol-ether extract prepared as described in the previous method, are pipetted into an Erlenmeyer flask of 50 cc. capacity. The ether is evaporated by heating on the steam bath, after which 0.5 cc. of the saturated aqueous solution of sodium hydroxide and sufficient redistilled alcohol to make a volume of approximately 30 cc. are added. The contents of the flask are boiled for 30 minutes and the alcohol then evaporated. The resulting residue is a mixture of brown and white material usually moistened with a few drops of water. The remaining water may be evaporated by means of a vacuum desiccator; in which event the subsequent benzene extraction will be colorless or nearly so. However, it is not necessary to dry the residue completely if the neutralization method is used, but it should be so dried for the oxidation method. After evaporation of the alcohol, the residue is treated with an amount of the aqueous HCl solution which represents 0.25 cc. more of the acid solution than is required to neutralize the 0.5 cc. of NaOH solution originally added to produce saponification. Then sufficient water is added to make the total addition of liquid equal to about 1.5 cc. The acid solution is mixed with the saponified residue by gentle rotation of the flask and heated on the steam bath until the brown colored material described above floats on the surface of the water in a liquid state. To the flask are now added about 15 cc. of benzene and the whole brought to boiling on the steam bath. The heating and frequent rotation of the flask are continued about 5 minutes and then the supernatant benzene decanted into a beaker of 100 cc. capacity. The process of extraction is repeated four times. The benzene is now evaporated, first on the electric hot plate nearly to dryness, then completed on the steam bath, finally the last traces of benzene are removed by placing in the vacuum desiccator for about 15 minutes.

The residue from the benzene extractions is extracted five times on the steam bath with redistilled petroleum ether. The petroleum ether extractions are decanted through a 7 cm. filter paper into a 125 cc. Erlenmeyer flask. The extract is then evaporated to dryness on the steam bath and the fatty acids estimated by either the neutralization or the oxidation method. If the latter is to be used the fatty acids must be separated from

cholesterol. This is accomplished by resaponifying and dissolving out cholesterol by the procedure described in the method for "combined fats and free fatty acids."

The quantitative accuracy of the various procedures and methods described has been established by carrying them out using known amounts of C.P. oleic acid as the free acid and after its addition to specimens of previously analyzed bile. The efficiency of the alcohol-ether extraction was determined by saponifying the precipitate formed on the addition of bile. The saponified precipitate contained negligible amounts of fatty acids. The efficiency of the CCl_4 extraction of the fats was determined as follows: The roll of filter paper was extracted with CCl_4 as described in the procedure for partitioning fats and fatty acids. It was then macerated by grinding in a mortar with sand, and again extracted with CCl_4. No fatty acid compounds were recovered. A series of comparative analyses of the same specimens of bile by the neutralization and oxidation methods gave results which agreed closely.

The bromine numbers of total fatty acids of the bile specimens and also of blood samples withdrawn during the periods of collection of duodenal contents are outlined in table 1.

It is emphasized that the oil was administered through the tube directly into the duodenum, the tube washed first with 50 cc. of water and then with bile before collection for analysis was begun. Furthermore, at least 15 minutes elapsed after oil administration before collection of bile for analysis was commenced. It is obvious that the oil has passed far beyond the region of the duodenal tube long before the collection of specimens for analysis had begun. Therefore, the total fatty acids of the duodenal bile specimens were not contaminated by the oil administered.

Study of the table shows that after cottonseed oil administration the bromine numbers were all well below that of the oil; which was 49.2. The bromine numbers of total fatty acids of four specimens of fasting bile varied from 28.2 (expt. 7) to 35.5 (expt. 3). Following food administration the extremes of bromine numbers were somewhat greater: i.e., 20.8 (expt. 1) and 37.0 (expt. 6). In three experiments (expts. 6, 7, 9) the bromine numbers of succeeding specimens were fairly uniform indicating comparable mixtures of fatty acids. In four experiments (expts. 1, 2, 3, 8) the bromine numbers of succeeding specimens varied sufficiently to show the presence of different mixtures of fatty acids.

In 5 specimens (expt. 2, period 1; expt. 3, periods 1 and 2; expt. 5, period 1; expt. 7, period 2) the bromine numbers of the total fatty acids of duodenal bile were sufficiently comparable to those of the blood to indicate that the mixtures of fatty acids from these two sources were probably similar. The 18 other bile bromine numbers were sufficiently different from those in the blood to show that the mixtures of fatty acids were dissimilar.

TABLE 1

Bromine numbers of total fatty acids of bile and of blood following administration of pure foodstuffs

EXPERIMENT	SPECIMEN	BROMINE NUMBER OF FATTY ACIDS		TEST MEAL
		Bile	Blood	
1	F*		25.3	
	1	34.5	23.7	Cottonseed oil
	2	20.8	19.8	
	3	26.5	24.1	
2	F		23.2	
	1	29.1	27.7	Cottonseed oil
	2		24.2	
	3		28.9	
3	F	35.5	31.7	
	1	31.6	29.1	Cottonseed oil
	2	32.4	29.8	
	3	24.6		
4	F	28.9	18.1	
	1	26.1	22.6	Beef peptone
	2		18.6	
5	F		28.2	
	1	29.1		Beef peptone
	2		28.7	
	3		32.2	
	4		24.5	
	5		30.0	
6	F		28.3	
	1	37.0	31.5	Casein peptone
	2	35.2	27.9	
	3		26.8	
	4		33.0	
7	F	28.2	31.2	
	1	28.9	25.1	Beef peptone
	2	29.3	31.9	
8	F		23.0	
	1	26.7	19.9	Dextrose
	2	32.1	28.5	
9	F		26.1	
	1	30.4	17.4	Dextrose
	2		22.1	
	3	30.2	22.3	
	4		23.2	
10	F	35.0	26.3	
	1	32.7		Dextrose
	2		21.1	

* F, fasting specimen.

In experiments 1 and 3 oil administration produced no increases in the bromine numbers of the blood over those of the fasting samples. In

experiment 2 the bromine numbers are so variable that an effect of oil administration is doubtful. The findings indicate that the ingestion of cottonseed oil had no influence on the bromine numbers of the fatty acids of the blood.

All the specimens of bile represented in table 1 were also analysed for the concentrations of both inorganic and organic phosphorus. The results obtained are outlined in the following representative table (table 2). Regardless of the volume of bile used for analysis the results outlined in the table are reduced to weights per cubic centimeter.

The findings as represented in table 2 showed that duodenal bile contained phosphorus in both organic and inorganic combinations. Increase in total phosphorus accompanied that of organic phosphorus, but an in-

TABLE 2

The organic and inorganic phosphorus content of duodenal bile following administration of pure foodstuffs

EXPERIMENT	SPECIMEN	MGM. PHOSPHORUS PER CC. OF BILE			TEST MEAL
		Organic	Inorganic	Total	
1	F*	0.025			
	1	**0.436**	**0.128**	**0.564**	Cottonseed oil
	2	**0.264**	**0.020**	**0.284**	
	3	0.054	0.021	0.075	
5	1	**0.151**	**0.119**	**0.270**	Beef peptone
	2	**0.056**	**0.080**	**0.176**	
	3	0.042	0.046	0.088	
	4	0.035	0.056	0.081	
8	F	0.037	0.047	0.084	
	1	**0.066**	**0.053**	**0.119**	Dextrose
	2	**0.071**	**0.056**	**0.128**	
	3	0.046			

* F, fasting specimen.

crease in the inorganic form was not constant. Comparison of these findings with those outlined in table 3 showed that increases in lipids were uniformly accompanied by increases in organic phosphorus, while increases in the inorganic were not so uniform. Following the administration of foodstuffs the organic form of phosphorus underwent the greater relative and actual increases in concentration. The findings were not quite complete enough to decide whether cottonseed oil is a more potent stimulant to phosphorus secretion than peptone. However, the results showed that both foodstuffs stimulated a considerable increase in the concentration of organic, and less of inorganic phosphorus in duodenal bile; while dextrose produced but a modest degree of stimulation in only one of three experiments. The findings were sufficiently distinctive to show that phosphorus

concentration in the bile is influenced differently by different types of foodstuffs.

The sources of fatty acids of duodenal bile, and their amounts calculated per cubic centimeter are outlined in table 3.

TABLE 3

The fatty acid content of lipid fractions of duodenal bile following administration of pure foodstuffs

EXPERIMENT	SPECIMEN	FATTY ACIDS OF LIPID FRACTIONS IN MGM. PER CC. OF BILE				TEST MEAL
		Fat	Free fatty acids	Soaps	Total fatty acids	
1	F*	0.18	0.19	0.32	0.69	Cottonseed oil
	1	**0.63**	**0.91**	**12.01**	**13.55**	
	2	0.19	**0.28**	**1.86**	**2.33**	
	3	0.00	0.34	0.53	0.87	
2	F	0.12	0.38	0.46	0.96	Cottonseed oil
	1	**0.44**	**1.27**	**7.75**	**9.36**	
	2	0.22	0.43	0.58	1.23	
	4	**0.19**	**0.63**	**2.23**	**3.05**	
3	F	0.45	0.30	0.39	1.14	Cottonseed oil
	1	0.42	0.17	**2.66**	**3.25**	
	2	0.43	**0.35**	**2.51**	**3.29**	
	3	**0.73**	**3.99**	**15.58**	**20.30**	
4	F	0.36	0.13	0.79	1.28	Beef peptone
	1	**0.82**	**0.39**	**1.67**	**2.88**	
5	1	0.38	0.19	**0.57**	**1.14**	
	2	0.34	0.27	0.08	0.69	
	3	0.28	0.34	0.04	0.66	
	4	0.33	0.22	0.19	0.74	
6	1	**0.50**	**0.90**	**0.35**	**1.75**	Casein peptone
	2	**0.20**	**0.69**	**0.14**	**1.03**	
7	F	0.25	0.13	0.90	1.28	Beef peptone
	1	**0.39**	**1.01**	**5.60**	**7.00**	
	2	**0.34**	**0.55**	**1.39**	**2.28**	
8	F	0.52	0.23	0.24	0.99	Dextrose
	1	**0.81**	0.14	**1.52**	**2.47**	
	2	**0.61**	**0.35**	**0.61**	**1.57**	
	3	0.27	0.16	0.14	0.57	
9	1	0.21	0.51	0.03	0.75	Dextrose
	2	0.25	0.27	0.10	0.62	
10	F	0.24	0.36	0.56	1.16	Dextrose
	1	0.29	0.28	0.73	1.30	
	2	0.29	0.09	0.33	0.71	

* F, fasting specimen.

Study of the table shows that the fatty acids of duodenal bile occurred as fats, soaps and free fatty acids. Excepting two experiments (expts. 9, 10; table 3) following administration of foodstuffs increased concentration of

the lipid fraction was found. Oil administration (expts. 1, 2, 3) was followed by the greatest and most prolonged concentration of fats, soaps and free fatty acids; peptone (expts. 4, 5, 6, 7) occupied second place; while dextrose (expts. 8, 9, 10) produced much less effect. Whenever increased concentrations of lipids occurred soaps were always increased, while in occasional experiments the fat (expt. 1, period 2; expt. 3, periods 1 and 2) or free fatty acids (expt. 3, period 1; expt. 8, period 1) fractions were not so affected. In experiments 5 and 6 no fasting contents were analysed, and the evidence of stimulation by foodstuffs of increased concentrations of lipids is the large amounts found in specimens just subsequent to the administration of food. Hence, the findings show three sources for the fatty acids of duodenal bile; and also that the concentration of lipids varies with the different types of foodstuffs administered.

DISCUSSION AND SUMMARY

In an unreported investigation (6) it was shown that the character of duodenal bile obtained after administration of cottonseed oil, peptone and dextrose reflected the immediate effect of these foodstuffs on the biliary functions of the liver. Therefore, the qualitative and quantitative changes in the lipid and the phosphorus fractions of the bile described in the present investigation show that these functional activities of the liver are also affected differently by different types of foodstuffs.

Fatty acids of duodenal bile occurred in the form of fats, soaps and free fatty acids; and phosphorus in both organic and inorganic combinations. These findings were anticipated because of similar observations (1) in bile derived from human gall bladders and operative fistulae. During the course of these observations (1) it was found that collecting duodenal bile in alcohol prevented demonstrable effects of enzyme action on bile lipids. These findings permit the conclusion that soaps, free fatty acids and inorganic phosphorus of human duodenal bile as well as fat and organic phosphorus reflect functional activities of the liver.

The frequency with which the admixtures of total fatty acids of bile and blood differed in their composition indicates that the lipids of bile are seemingly the result of secretory activity of the liver rather than a mere filtration product from the blood.

In a previous communication (6) it was demonstrated that the digestion and absorption of cottonseed oil is a powerful stimulant to the secretion of concentrated bile. In the present investigation the great increases in concentration of constituents of the bile following administration of oil show that the latter was absorbed. Nevertheless, sufficient oleic acid did not accumulate in the blood stream to influence the bromine numbers of the total fatty acids. This finding confirms a previously reported conclusion (7) which was that increases in the total fatty acids of the blood observed

after feeding fats to man were not the result of accumulation in the blood of the recently absorbed fatty acids.

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STUDIES ON THE EFFECTS OF HIGH OXYGEN PRESSURE

I. Effect of High Oxygen Pressure upon the Carbon-Dioxide and Oxygen Content, the Acidity, and the Carbon-Dioxide Combining Power of the Blood*

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Paul Bert (1878) showed conclusively that high tensions of oxygen produce toxic effects. In animals exposed to pressures above 3 to 4 atmospheres, the most striking characteristic was the appearance of convulsions which were rapidly fatal. Bert also found, in animals exposed to pressures of oxygen as low as 1 atmosphere, that the oxidation processes in the tissues were decreased with a consequent fall in body temperature. From these findings he concluded that oxygen in tensions above 1 atmosphere acted as a poison.

J. L. Smith (1899) observed that oxygen in increased tensions acted as an irritant and produced inflammation of the lungs. He found that while pulmonary damage occurred with tensions as low as 73 per cent of an atmosphere of oxygen, convulsions did not occur (in birds) below 2.7 atmospheres. He also noted the variability in the reactions of different animals in that larks developed convulsions at 3 atmospheres, while mice did not respond in a similar manner until the tension had been raised to 4.5 atmospheres.

Subsequent investigators have confirmed the essential findings of Bert and J. L. Smith and have defined more sharply the limits for the appearance of the symptoms of oxygen poisoning.

Hill and Macleod (1903a) observed that oxygen at or above 1 atmosphere pressure, lessened the carbon dioxide output and lowered the body temperature in mice, rats, and young rabbits. In animals exposed to 1.5 atmospheres of oxygen, inflammation and consolidation of the lungs were produced in 24 hours. The higher the oxygen tension, the more rapidly the changes ensued; e.g., 6 atmospheres of oxygen produced marked congestion in 6 hours. They noted that the convulsions (in mice, rats, and rabbits) were preceded by cleaning movements, salivation, gaping, and jerky, deep respiration. These convulsions were followed by coma and occurred frequently on exposures between 4 and 5 atmospheres of oxygen.

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In exposures of 6 to 25 atmospheres, coma usually supervened before the appearance of convulsions; while at 50 to 60 atmospheres, all forms of animal life were immediately convulsed and paralyzed.

Karsner (1916) studied the pathological effects of 80 per cent of an atmosphere of oxygen on rabbits. The only changes that could be attributed to the direct action of oxygen occurred in the lungs. At the end of 24 hours' exposure there was little change. In 48 hours either no changes occurred or changes which led to the development of marked congestion, edema, and early fibrinous bronchopneumonia. Three days' exposure led constantly to the development of a bronchopneumonia characterized by inflammatory changes in the bronchi, and desquamation of the alveolar epithelium associated with fibrinous exudation into the alveoli.

Binger, Faulkner, and Moore (1927) showed that dogs in an atmosphere of 70 to 80 per cent oxygen, after exposure of 3 to 7 days refused food, vomited, became drowsy, and were subject to respiratory distress characterized by slow, labored, deep breathing. They concluded that the dyspnea, cyanosis, and death occurring in some of their animals resulted from oxygen want due to destructive lesions in the lungs similar to those found by Karsner.

Campbell (1927) found that cats exposed to 40 per cent of an atmosphere of oxygen developed, after 18 to 27 days, a loss of appetite, sleepiness, and a loss in weight, but that monkeys, guinea pigs, rats, mice, and rabbits tolerated 60 per cent of an atmosphere of oxygen for prolonged periods without loss in weight.

More recently, F. J. C. Smith and his associates (1932) found that 83.6 per cent of an atmosphere of oxygen produced an active hyperemia and edema in the lungs of adult rats with a mortality of 13 per cent in 3 days. In the surviving rats, the pulmonary epithelium underwent hypertrophy and hyperplasia associated with a thickening and hyalinization of the pulmonary vessels. Moreover, these rats lost weight, their growth was impaired, and litters born during the exposure were premature and died during the first 24 hours.

Shilling and Adams (1933), working with rats, clearly defined the pressures of oxygen necessary for the production of convulsions in these animals in a 2-hour period. Thus no convulsions occurred below 3 atmospheres. At 4 atmospheres, 50 per cent of the rats had convulsions; while at 4.5 atmospheres, all of the rats developed convulsions in the 2-hour exposure. Moreover, the higher the pressure, the more rapid the average time of onset of the convulsions. At autopsy, the lungs were edematous and contained areas of hemorrhagic exudation.

The characteristics, then, of acute oxygen poisoning in animals as revealed by the literature are as follows:

1. Convulsions, which appear after exposure to tensions above 3 to 4 atmospheres. The higher the tension, the more rapid the onset of convulsions and the sooner the occurrence of death.

2. Pulmonary damage, which occurs after exposures to tensions above 70 per cent of an atmosphere. The pulmonary changes vary in character and intensity depending on the tension of the oxygen and the duration of the exposure. In oxygen tensions above 4 atmospheres, death occurs either as a result of the convulsions or from a combined effect of the oxygen on the nervous system and the lungs. In tensions below 3 atmospheres, death is usually the result of pulmonary damage.

3. Decreased metabolism, which has been measured as a lessened carbon-dioxide output or, in some cases, as a decreased oxygen consumption. Fall in body temperature has been associated with this change.

The characteristics of chronic oxygen poisoning appear in animals which survive for prolonged periods in tensions of oxygen slightly in excess of 70 per cent of an atmosphere. They may be listed as anorexia, drowsiness, loss in weight, impaired growth, and in the litters of pregnant rats, premature birth and early death. These changes together with variations in respiration are secondary to the striking pulmonary alterations, which are characterized by hypertrophy and hyperplasia of the epithelium associated with a thickening and hyalinization of the blood vessels.

With reference to man, Bornstein (1910) breathed oxygen at 3 atmospheres pressure for periods up to 48 minutes without distress. He extended his experiments to caisson workers during decompression and found that the men could breathe oxygen with impunity at 3 atmospheres for periods up to 30 minutes. In 1912, Bornstein and Stroink undertook an experiment in which the former, while exercising on a bicycle, breathed oxygen at 3 atmospheres. After 51 minutes spasms occurred in the muscles of the legs. These subsided when air was substituted for oxygen. Bornstein considered these spasms to be a manifestation of mild oxygen poisoning. On the basis of these experiments, the limit for the safe breathing of oxygen at 3 atmospheres pressure was placed at approximately 30 minutes.

All previous experiments with oxygen tensions above 1 atmosphere have been accomplished by placing the animals in small pressure chambers, where they were beyond the immediate control of those conducting the experiments until the pressure had been lowered again to atmospheric. Moreover, in these experiments it was often difficult if not impossible to control temperature and humidity changes. While convulsions and pulmonary damage could be studied profitably under these conditions, measurements of metabolism, respiration, and circulation, together with the withdrawal of blood for blood gas and chemical analyses, involved complicated technique which was subject to many errors. Thus the frothing

of the blood, due to the effervescence of dissolved gases, which occurred when blood was withdrawn from an animal under pressure, would interfere with quantitative and perhaps qualitative studies.

In the following series of experiments, the technical difficulties have been lessened by utilizing a pressure chamber sufficiently large so that the individuals conducting the experiments could be accommodated. In this way the methods of procedure were essentially the same as those which would be employed in the laboratory at atmospheric pressure.

The purpose of these studies is to record the effects of 4 atmospheres of oxygen for periods of from 2 to 4 hours on dogs anesthetized with sodium diethylbarbiturate (Barbital-Sodium, Merck). In this paper we shall discuss the blood gases, carbon-dioxide combining power, and the blood acidity. In subsequent papers of this same series we shall present our observations on the changes in the chemical constituents of the blood, the metabolism, blood pressure, respiration, neuromuscular activity, and the pathologic findings.

METHOD. The animals used in these experiments were dogs weighing between 16 and 25 kgm. Sodium diethylbarbiturate dissolved in 50 cc. of isotonic salt solution was administered intraperitoneally. The usual amount given was 0.33 gram per kilogram of body weight.

The experiments were carried out in one of the compartments (12 feet long and 8 feet in diameter) of the pressure chamber described by Thomson, Yaglou, and Van Woert (1932). The pressure was held at 45 pounds gauge, which is equivalent to 4 atmospheres absolute pressure. Two of the authors remained in the chamber throughout the experiments. A cannula was inserted in the trachea and connected to a spirometer filled with pure oxygen. The spirometer used was the standard 6-liter Benedict apparatus in which oxygen is rebreathed, the carbon dioxide being absorbed through a soda-lime cartridge. At intervals the spirometer was rinsed in order to eliminate the dissolved nitrogen from the animal's body. Analyses of the spirometer gas showed the oxygen concentration to be constantly 97 per cent. At a gauge pressure of 45 pounds, therefore, the dog breathed oxygen having a partial pressure of about 3.88 atmospheres or 2950 mm. Hg.

Approximately 2 hours after the administration of the anesthesia and while the dog was still breathing room air at normal pressure, samples of arterial and venous blood were taken. The arterial blood was withdrawn without contact with air from a cannula inserted in the femoral artery and introduced into an oxalated blood-collecting tube over mercury. The mixed venous blood was handled in a similar manner and was drawn from the right side of the heart by means of a glass tube which was inserted through the external jugular vein. The chamber was then closed and the pressure raised to 4 atmospheres. After the dog had breathed oxygen at

this pressure for periods varying from 52 to 193 minutes, a second series of blood samples was taken for comparison with the first series. Blood pressure, temperature, and respiration were recorded throughout the experiments.

We have estimated that when 100 cc. of blood are equilibrated at a temperature of 39°C. with pure oxygen at 1 atmosphere, approximately 1.9 cc. of oxygen will be taken up in physical solution. This calculation is based upon the solubility of oxygen in water, of which the blood contains approximately 79 per cent. At 4 atmospheres pressure, 100 cc. of blood, then, will take up 7.6 (4 × 1.9) cc. of oxygen. Therefore, when the pressure falls from 4 atmospheres to 1 atmosphere, the dissolved oxygen will come out of solution. In order, then, to determine the oxygen content of the blood drawn under pressure, it was necessary to take our samples into calibrated syringes and then deliver the entire content, including both the blood and the effervescing gas, into the Van Slyke apparatus. For this purpose a 3-cc. syringe was used (fig. 1) with a brass attachment through which passes an adjustable pin. This pin was then locked by 2 wing nuts, acting in opposite directions, in such a position that when the plunger was drawn against the end of the pin the delivery of the syringe was approximately 1 cc. The exact delivery was determined by calibration and the necessary corrections applied. After filling the syringe with blood, the tip was sealed off with paraffin and the adjusting pin released so that the plunger could move freely in response to the pressure of the oxygen coming out of solution. To deliver the blood sample from the syringe into the Van Slyke apparatus, the paraffin seal was removed from the tip of the syringe and its contents were then drawn into the apparatus under sufficient negative pressure to evacuate both the tip and the dead space of the syringe of its contents. The blood samples collected under atmospheric pressure were delivered into the apparatus by means of a pipette.

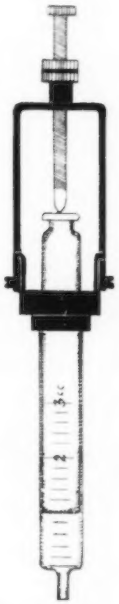

Fig. 1. Syringe for taking blood samples under high gas pressures.

The blood samples were kept at a temperature of 4.0°C. until analyzed. The analyses were made with the standard Van Slyke apparatus (closed manometric) by the methods of Van Slyke and Neill (1924). Using this method check determinations for both carbon dioxide and oxygen showed a maximum error of ±0.15 volume per cent. The blood samples collected under pressure and delivered from a syringe showed a maximum error of ±0.5 volume per cent.

The carbon-dioxide capacity of the blood was determined from the

carbon-dioxide content of arterial blood equilibrated at a temperature of 39°C. with 2 gas mixtures each containing oxygen at a tension of 190 mm. but with carbon dioxide at a tension of 40 mm. in one mixture and 70 mm. in the other. This established two points on the carbon-dioxide absorption curve. From these data the curve for arterial blood was plotted logarithmically after the method of Peters (1923), who showed that the graph when plotted in this manner became a straight line. The carbon-dioxide absorption line for venous blood runs parallel to the arterial line but at a higher level. Dill has shown that the difference in level is a function of the carbon dioxide capacity, the oxygen capacity, and the per cent saturation of the hemoglobin with oxygen, and may be accurately calculated from a chart based on these factors. From the carbon-dioxide absorption graph constructed in this manner, the carbon-dioxide tension of the blood may be determined from the carbon-dioxide content. The maximum error in estimating the carbon-dioxide tension of the blood drawn at atmospheric pressure was 1.1 mm., and for blood drawn under 4 atmospheres oxygen pressure, it was 2 mm. The difference is due to the greater accuracy in measuring the volume of a blood sample when delivered from a pipette as compared with delivery from a syringe.

The pH of the blood was calculated from the general Henderson-Hasselbalch equation: $pH_s = pK' + \log \frac{BHCO_3}{H_2CO_3}$. This calculation is based upon the carbon-dioxide content of the plasma. We found it more convenient to use whole blood and then to calculate the carbon-dioxide content of the plasma from the whole-blood content according to the method of Van Slyke, Sendroy, and Liu (1932). The precision of this method depends upon the accuracy of four values: the carbon-dioxide content, the carbon-dioxide tension, the constant pK′, and the solubility constant of H_2CO_3. When the errors of these four values are maximal and in the same direction which is to be expected only occasionally, the total maximum error will be 0.034 pH for blood drawn at atmospheric pressure, and 0.038 for blood drawn under 4 atmospheres of oxygen pressure.

Experimental results. The experimental data are given in table 1. The oxygen content of the arterial blood taken while the dog was breathing oxygen at pressures of from 3 to 3.89 atmospheres was, in all but one case, in equilibrium with the oxygen tension in the lungs. This fact was established by comparing the observed volume of oxygen carried in physical solution with the volume which the blood would carry if it were in equilibrium with the oxygen tension in the lungs. The observed volume per cent of free oxygen, or oxygen in physical solution, is equivalent to the total oxygen minus the oxygen bound by the hemoglobin. The calculated volume per cent of dissolved oxygen is equivalent to the oxygen tension in the lungs expressed in atmospheres multiplied by 1.9. The oxygen tension

in the lungs (see column 12, table 1) was the same as the oxygen tension in the spirometer minus the water vapor pressure (52 mm.) and the carbon-dioxide tension (approximately 45 mm.) in the lungs.

As an example of the above calculations let us cite experiment 17. A sample of blood was drawn while the dog was breathing oxygen at a pressure of 3.84 atmospheres and equilibrated with 25 per cent oxygen. The oxygen capacity of the arterial blood equilibrated with 25 per cent oxygen was 19.56 volumes per cent, of which 0.47 (0.25×1.9) volume per cent was in physical solutions. Therefore, the combined oxygen was 19.09 ($19.56 - 0.47$) volumes per cent. The oxygen content of the arterial blood was 26.04 volumes per cent. The difference, 6.95 ($26.04 - 19.09$) volumes per cent, represents the oxygen in physical solution. Since the oxygen pressure in the lungs was 3.72 atmospheres and the blood took up 1.9 volumes per cent of oxygen in physical solution per atmosphere of oxygen pressure, the calculated value for free oxygen was 7.07 (1.9×3.72) volumes per cent. Thus the difference between the observed and the calculated free oxygen is only 0.12 volume per cent.

With the exception of experiment 25, it will be noted that the difference between the observed and the calculated volumes of oxygen in physical solution in arterial blood agrees within 2.7 per cent (table 1, columns 9, 10, and 11). In experiment 25, the exposure to oxygen was unusually long, resulting in lung damage which undoubtedly hindered the free diffusion of oxygen into the blood. In the absence of such injury, we may conclude that the arterial blood is in equilibrium with the oxygen tension in the lungs.

The oxygen tension of the blood drawn from the right side of the heart is a fair indication of the average oxygen tension of the tissues. In column 14, table 1, are given the oxygen tensions in the mixed venous blood drawn while the dog was breathing oxygen under pressure. If the oxygen content of the venous blood was less than that found in the blood equilibrated with 25 per cent oxygen, as was the case in experiments 18, 21, 22, and 25, it is fair to assume that the oxyhemoglobin was not 100 per cent saturated. In such cases the oxygen tension was calculated from the oxygen absorption curve of the oxyhemoglobin, which correlates oxygen tension with per cent oxygen saturation. Notwithstanding the fact that the arterial tension in the arterial blood varies but little in the different experiments, there is a very wide range of variation in the oxygen tension of the mixed venous blood. This discrepancy may be attributed to individual variations in the circulatory rate and the metabolic rate of the dogs. A high metabolic rate or a slow circulatory rate will tend to reduce the oxygen tension in the blood passing through the tissues, and a low metabolic rate or a rapid rate of circulation will have the opposite effect. In the same manner the oxygen tension in the different tissues of the same animal will vary in accordance

TABLE 1

		O_2 CONT.		O_2 CAP.		CO_2 CONT.		CO_2 AT CAP. AT 40 MM		O_2 IN PHYSICAL SOLUTION						p CO_2		pH	
										Arterial				Venous					
		A	V	A	Diff.	A	V	A	Diff.	Obs.	Cal.	Diff.	pO_2	Obs.	pO_2	A	V	A	V
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		vol. %	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*vol. %*	*mm.*	*vol. %*	*mm.*	*mm*	*mm.*		
14	Air = 1 Atmos.			19.58				42.5											
	pO_2 = 3.0, 132 min.	25.61	20.36	20.47	+0.89	45.91	51.74	43.9	+1.4	5.47	5.61	−0.14	2190	0.36	130	44.5	59.7	7.35	7.26
17	Air = 1 Atmos.	20.47	12.05	20.32		44.30	49.13	41.0								47.0	54.0	7.33	7.30
	pO_2 = 3.84, 100 min.	26.04	20.48	19.56	−0.76	42.34	48.50	40.0	−1.0	6.95	7.07	−0.12	2827	1.39	555	45.0	60.0	7.35	7.28
18	Air = 1 Atmos.			23.07				40.2											
	pO_2 = 3.36, 64 min.	28.86	21.58	23.04	−0.03	39.92	44.28	40.2	0.0	6.29	6.16	+0.13	2462		80	39.0	48.0	7.36	7.31
19	Air = 1 Atmos.	19.0	14.25	19.06		42.38	45.50	43.8								37.0	41.0	7.40	7.37
	pO_2 = 3.92, 67 min.	25.1	20.14	18.39		42.45	47.0	45.0	+1.2	7.18	7.22	−0.04	2888	2.22	880	34.0	45.0	7.43	7.34
20	Air = 1 Atmos.	21.68	16.70	23.35		45.27	48.60	41.5								48.0	51.0	7.32	7.31
	pO_2 = 3.84, 193 min.	30.87	25.60	24.44	+1.11	38.80	41.70	41.0	−0.5	6.90	7.07	−0.17	2827	1.63	645	35.0	41.5	7.41	7.36

21	Air = 1 Atmos.	21.31	15.77	23.76		51.70	55.22	44.2								54.5	58.5	7.33	7.31
	pO_2 = 3.89, 92 min.	31.94	24.51	25.40	+1.64	50.91	55.52	44.2	0.0	7.01	7.16	−0.15	2865		90	55.0	67.0	7.32	7.26
22	Air = 1 Atmos.	23.68	19.60	25.80		32.85	37.64	39.5								24.8	31.5	7.50	7.47
	pO_2 = 3.88, 119 min.	31.47	24.58	24.63	−1.17	39.11	46.33	39.2	−0.3	7.31	7.14	+0.17	2857		160	40.0	59.0	7.36	7.27
24	Air = 1 Atmos.	20.30	18.88	22.44		45.0	46.55	44.25								45.0	48.0	7.34	7.33
	pO_2 = 3.89, 62 min.	29.27	25.90	22.77	+0.33	44.0	46.63	44.25	0.0	6.97	7.16	−0.19	2865	3.60	1440	43.5	50.0	7.35	7.31
25	Air = 1 Atmos.	18.9	13.64	20.82		43.86	46.15	43.0								40.0	41.5	7.38	7.38
	pO_2 = 3.88, 165 min.	24.55	16.22	20.80	−0.02	41.38	48.59	43.5	+0.5	4.22	7.16	2.94			45	35.0	47.0	7.41	7.35

with the metabolic activity and the blood supply to those tissues. It is significant that when the oxygen requirements of the body are being satisfied by the oxygen carried in physical solution, then an increase of 1 volume per cent in the free oxygen content of the blood will indicate that the blood is under an increased oxygen tension of 400 mm. Hg.

The average carbon-dioxide tension while the dogs were breathing room air, was 42.3 mm. in the arterial blood, and 46.5 mm. in the venous blood. While breathing oxygen under pressure, the average carbon-dioxide tension in the arterial blood was 41.2 mm., and in the venous blood, 53 mm. (columns 15 and 16, table 1). The capacity of the blood drawn during the inspiration of room air, to combine with carbon dioxide at a tension of 40 mm. varied from 39.5 to 44.2 volumes per cent (column 7, table 1). A comparison of the combining capacity of blood drawn during exposure to room air and that drawn during exposure to oxygen under pressure showed a difference so slight as to be negligible and in most cases well within the experimental error.

The arterial carbon-dioxide tension was subject to wide variations from normal due to irregularities in the respiratory volume. In many cases the respiratory rhythm was altered by the anesthesia and caused either an increase or a fall in the rate.

The average pH of the normal arterial blood was 7.35, and of the venous blood 7.33, not including experiment 22 in which sustained hyperpnea occurred. During the inhalation of oxygen under pressure the average pH of the arterial blood was 7.37, and fell to 7.30 in the venous blood. It is significant that whereas blood at atmospheric pressure carried its load of carbon dioxide from the tissues to the lungs with a fall in pH of only 0.02, there was a fall of 0.07 in the pH of the blood which was exposed to the pressure of oxygen sufficiently high so that the oxygen requirements were satisfied by the oxygen in solution without the reduction of the oxyhemoglobin.

The sodium diethylbarbiturate anesthesia undoubtedly tends to modify the convulsions of oxygen poisoning. Instead of the generalized convulsions as described by Shilling and Adams (1933), there occurred a violent spasm which was confined to the muscles of the head, neck, and thorax, and which was accompanied by a rapid inspiration. As a rule the lower part of the trunk and the extremities were not involved. The duration of the spasm was a matter of a few seconds during which there was a vigorous shaking of the head, neck, and chest. At times these parts were thrown off the dog board by the violence of the muscular contractions. Relaxation occurred abruptly after each spasm and was accompanied by expiration. Each seizure represented a respiratory cycle, and the number of these convulsive movements increased and wholly replaced the original respiratory rhythm. These convulsions, then, consisted essentially of con-

vulsive respiration which terminated in an apnea during which circulatory failure gradually ensued. In experiments 18 and 24, table 1, convulsions occurred. In experiment 24, the oxygen pressure was maintained and death terminated the convulsive state; while in experiment 18, air replaced oxygen and the original respiratory rhythm was resumed.

The pulmonary damage was characterized consistently by marked congestion and varying degrees of atelectasis. The bronchial tree, however, was dry and there was no hemorrhage into either the tissues or the alveoli. The latter were remarkably free from exudate.

DISCUSSION OF RESULTS. *The dissolved oxygen in the arterial blood.* J. L. Smith (1897–1898) found that under a tension of 1.87 atmospheres for as short a period as 3 hours, the power of the lungs (of mice) to absorb oxygen was diminished. He believed that the pulmonary inflammation protected the nervous system from the toxic effects of oxygen by lowering the tension of this gas in the arterial blood.

In our experiments the close correspondence between the calculated and the observed values for dissolved oxygen in the arterial blood, indicates that equilibrium between the oxygen tension in the lungs and the oxygen tension in the blood has been attained. The passage of oxygen, therefore, from the lungs into the blood takes place by simple diffusion and conforms to Henry's law. In but one experiment (25) was the observed oxygen tension lower than the calculated value. As a rule, however, we may conclude that the pulmonary damage which occurs after 3 hours' exposure to 4 atmospheres of oxygen will not prevent equilibrium from being reached between arterial and alveolar oxygen.

The oxygen capacity of the blood. No change occurred in the capacity of the hemoglobin to take up oxygen as a result of exposure to high oxygen pressure. The variations, such as an increase of 1.64 volumes per cent in experiment 21, and a decrease of 1.17 volumes per cent in experiment 22, are due probably to changes in the cell content of the blood. The majority of the changes are so small as to be within the range of experimental error.

The carbon-dioxide combining power of the blood. Bean and Haldi (1932) found an increase in lactic acid in the blood of dogs which were subjected to pressures of oxygen between 4 and 5 atmospheres for periods up to 30 minutes. They considered that decreased oxidation as a result of the increased oxygen tension (Bert, 1878; Hill and Macleod, 1903a, b, c; and Bean, 1931) was a probable explanation; that, although there was an abundance of oxygen in solution, the capacity of the tissues to utilize this oxygen was impaired, and as a result lactic acid accumulated.

In our experiments, the carbon-dioxide combining capacity of the blood drawn while the dog was breathing air at atmospheric pressure was almost identical with the carbon-dioxide capacity of the blood drawn under 4 atmospheres of oxygen pressure. If increased amounts of lactic acid had

been formed during exposure to high oxygen pressure, we should expect to find a fall in the carbon dioxide combining capacity at the rate of 1 volume per cent of carbon dioxide for every 4.04 mgm. per cent of lactic acid increase. The fact that there was no significant change in the carbon-dioxide combining power leads us to infer that if decreased oxidations occur, they do not result in the increased production of lactic acid or other organic acids.

Increased acidity as a factor in oxygen poisoning. In recent work (Gesell, 1923; Campbell, 1929–1930; and Bean, 1931, 1932), increased acidity in the blood and tissues has been stressed as an important factor in the production of the symptoms of oxygen poisoning. The cause of this increased acidity, according to Gesell (1923), is due to the fact that at approximately 3 atmospheres of oxygen pressure, the tissue requirements are satisfied by the oxygen in physical solution. Hence the hemoglobin is not reduced in its passage through the capillaries. Since three-quarters of the alkali which is liberated in the blood to buffer and carry the carbon dioxide is derived from the reduction of oxyhemoglobin, the transport of carbon dioxide would be markedly disturbed and a tissue acidosis would result. Campbell (1929) gave support to this theory in a series of experiments with rabbits in which it was shown that the carbon-dioxide tension in the tissues (measured by analyses of nitrogen injected under the skin and into the abdominal cavity) rose to dangerous levels at oxygen pressures of from 2 to 5 atmospheres. In 4 out of 5 animals the carbon-dioxide tension was 213 mm. Hg (30 per cent) or more, whereas 4 controls in air at the same pressure developed an average carbon-dioxide tension of 86 mm. Bean (1931) found a rise in acidity of the blood in 21 out of 23 dogs subjected to oxygen pressures between 4 and 5 atmospheres. This increase in acidity varied from 0.05 to 0.19 pH. Bean accepts Gesell's theory in explaining his results.

While we grant that an increase in acidity in the venous blood is possible when the oxyhemoglobin is not reduced, we believe that this increase in acidity is actually so small as to be insignificant as an etiological factor in oxygen poisoning. Our conclusion is based upon the following calculations and experimental results.

1. According to Peters and Van Slyke (1931, pp. 539–540) the complete reduction of the oxyhemoglobin enables the blood to carry 0.7 mol of H_2CO_3 per mol of oxygen exchanged, without any alteration of pH. They have conveniently represented this by the equation: $K_2HbO_2 - O_2 + 0.7\ H_2CO_3 = K_{1.3}H_{0.7}Hb + 0.7\ KHCO_3$. Although oxyhemoglobin is unreduced as under high oxygen pressures, it can still act as a buffer and transport carbon dioxide but a lowering of the pH will occur. With a change of 1 pH unit, 2.5 equivalents of acid can be added to one mol of oxyhemoglobin. Therefore, the 0.7 mol of H_2CO_3 which is carried without change in pH for

every mol of oxyhemoglobin completely reduced, would be carried (if the oxyhemoglobin were not reduced) with an increased acidity of the venous blood of $0.7/2.5 \times 1$ pH unit or 0.28 pH. However, in animals at rest the oxyhemoglobin is usually not more than 30 per cent reduced, so that the actual increase in acidity would not be more than 30 per cent of 0.28, or 0.084 pH. Moreover, the action of the other blood buffers such as the bicarbonate and the plasma proteins might modify this change in pH from 0.084 to 0.05. From these figures it is seen that the change in acidity as represented by the pH may be comparatively small.

2. If we grant that the oxyhemoglobin must carry a given load of carbon dioxide at a higher tension than reduced hemoglobin, it is a simple matter to estimate this rise in pressure from a study of the carbon-dioxide absorption curves of reduced and oxygenated blood. If the venous blood of an animal in the resting state carries an increase of 5 volumes per cent of carbon dioxide over that in the arterial blood, and if the oxyhemoglobin is reduced one-third, then the difference in tension between the venous and the arterial blood will be about 6 mm. If, on the other hand, the oxyhemoglobin of the venous blood remains unreduced as is the case when high tensions of oxygen are inspired, the 5 volumes per cent of carbon dioxide will be carried with a tension difference of 12 mm. between the venous and the arterial blood. Thus, it is observed that there may be only an increase of 6 mm. in the carbon-dioxide tension when the oxyhemoglobin remains unreduced.

3. Our experimental results agree very closely with the above calculations. Thus the average venous-arterial carbon-dioxide tension difference during the inspiration of room air was 4.2 mm., and during the inspiration of oxygen at approximately 4 atmospheres pressure, 11.8 mm. The average carbon-dioxide tension of the mixed venous blood is only 6.5 mm. higher and the pH only 0.03 unit lower as a result of the change from air at atmospheric pressure to oxygen at a pressure of 4 atmospheres. Such data tend to preclude increased acidity as a factor in the production of the major symptoms of oxygen poisoning.

4. Moreover, the maximum acidity resulting when oxyhemoglobin is not reduced, occurs as soon as the oxygen in physical solution is just sufficient to satisfy the tissue requirements. This may occur when the oxygen pressure is as low as 3 atmospheres (Bean, 1931) or, as in our experiments, slightly below 4 atmospheres. If the symptoms of oxygen poisoning were due to the increased acidity resulting when oxyhemoglobin is not reduced, they should be well developed at these pressures. This, however, is not the case. In our experiments only 2 of the 9 dogs developed convulsions, and in each case the change in acidity under the increased oxygen tension was so small as to be without significance in so far as the etiology of the convulsions is concerned.

This leads us, then, to a brief consideration of the relationship of the oxygen tension to the production of convulsions. From a study of our experiments and from a review of the literature (Bert, 1878; J. L. Smith, 1899; Hill and Macleod, 1903c; Bornstein and Stroink, 1912; Hansen, 1925; Shilling and Adams, 1933) it appears that at 3 atmospheres oxygen pressure a borderline exists in which the oxygen tension in the tissues (of the experimental animals) is not sufficiently high to produce convulsions. At 4 atmospheres, from 20 to 50 per cent of the animals will develop convulsions. At 5 atmospheres, the dissolved oxygen will be raised beyond the limits of the factors producing variation. At this pressure convulsions should occur in all experiments.

As to the exact mechanism by which this increased oxygen tension operates to produce the convulsions and the metabolic and circulatory changes of acute oxygen poisoning, we know little more than Paul Bert (1878, p. 801) who stated that, "Oxygen acts like a rapidly toxic poison when its quantity in the arterial blood is raised to about 35 cc. per 100 cc. of blood," and (p. 1136), "I do not know anything in physiological chemistry more curious than this type of action of the dissolved oxygen, having as its result, not to activate but to stop a combination. Although there may be possible explanations, it is certain that the organic oxidations do not continue when the blood cells, saturated with oxygen, are surrounded by free oxygen dissolved in the plasma and the tissues."

As to the mechanism of the pulmonary damage, we can say little more than J. L. Smith (1899) who stated that oxygen at high tension acted as an irritant or pathological stimulant and produced inflammation.

It may be recognized, however, that two distinct processes are occurring in oxygen poisoning. The one, due to the increased tension of oxygen in the blood, which may affect the nervous system so profoundly that convulsions result; the other, due to the direct action of the alveolar oxygen on the pulmonary epithelium which produces inflammatory changes. At oxygen tensions above 3 atmospheres, the former process may overshadow the latter, while at lower tensions, the pulmonary inflammation dominates the picture. At tensions around 80 per cent of an atmosphere, in animals surviving the early pulmonary changes, there will appear the symptoms of chronic oxygen poisoning such as anorexia, loss in weight, and impaired growth—symptoms which are probably secondary to the pulmonary damage. In the words of Campbell (1927), it might be conceived in the above cases that some poison was formed by the action of the oxygen upon the lung epithelium and that a general poisoning was the result.

SUMMARY AND CONCLUSIONS

In dogs anesthetized with sodium diethylbarbiturate administered intraperitoneally and subjected to 4 atmospheres of oxygen pressure for periods of from 2 to 4 hours, the following results were obtained:

Convulsive seizures occurred in 2 out of 9 experiments and were characterized by spasmodic respiration.

The pulmonary damage consisted uniformly of congestion and varying degrees of atelectasis. Hemorrhage or edematous exudation into the alveoli or bronchi did not occur.

The tension of oxygen in the arterial blood was equal to the tension of oxygen in the alveolar air, indicating equilibrium and the absence of any protective mechanism in the lungs against the high oxygen tension.

The arterial blood contained approximately 7 cc. of oxygen in solution per 100 cc. of blood, sufficient in most cases to satisfy the oxygen requirements of the tissues without reduction of the oxyhemoglobin.

The oxygen tension of the mixed venous blood varied widely due probably to variations in the circulatory and metabolic rates of the animals.

The carbon-dioxide combining capacity of the blood was unaltered by the inspiration of oxygen at 4 atmospheres, indicating the absence of increased lactic acid or other organic acids.

The average carbon-dioxide tension of the blood during the inspiration of room air was: arterial, 42.3 mm.; and venous, 46.5 mm. During the inspiration of oxygen at 4 atmospheres, the average carbon dioxide tension was: arterial, 41.2 mm.; and venous, 53 mm.

The average pH of the blood during the inspiration of room air was: arterial, 7.35; and venous, 7.33. During the inspiration of oxygen at 4 atmospheres, the average pH was: arterial, 7.37; and venous, 7.30.

It seems highly improbable that the rise in carbon-dioxide tension due to the absence of reduction of the oxyhemoglobin, and the resultant increase in acidity is sufficient to account for the symptoms of oxygen poisoning.

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STUDIES ON THE EFFECT OF HIGH OXYGEN PRESSURE

II. Effect of High Oxygen Pressure on the Sugar, Phosphorus, Non-protein Nitrogen, Chloride, Creatinin, Calcium and Potassium Content of the Blood*

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The necessity for a re-investigation of the effects of increased oxygen tension of mammals has been pointed out in the introduction to the first of this group of papers (Behnke, Shaw, Shilling, Thomson and Messer, 1933). The two most noticeable effects of exposure to high oxygen tensions are the convulsions and pulmonary damage; and as shown by Behnke and his associates, confusion exists as to the exact mechanism producing these changes.

The present paper reports the results of an examination of the chemical composition of the blood in dogs exposed to oxygen at pressures of four atmospheres, and includes the following determinations: sugar, phosphorus, non-protein nitrogen, chloride, creatinin, calcium, and potassium on the blood; sugar, protein and cell count on the cisternal fluid; specific gravity, reaction, albumen, sugar and acetone on the urine. The lactic acid studies on these dogs will be reported in a subsequent publication.

Technique. The technique of the experiment has been described in the first paper of this series (Behnke et al., 1933). In the present study 20 dogs, ranging in weight from 14.3 to 26.5 kilos, were used. Three were used as controls, breathing air at atmospheric pressure; 16 were exposed for from 2 to 4 hours to 97 per cent oxygen at 45 pounds gauge pressure, and 1 at 60 pounds gauge pressure. The animals were given 0.33 gram of sodium diethyl-barbiturate (Barbital-Sodium, Merck) per kilo intraperitoneally. It cannot be too strongly stressed that the conditions for all experiments were maintained constant. This was made possible, first, by the construction of the chamber and second, by the presence of at least two experimenters under pressure in the chamber with the animal at all times.

The first blood sample was always taken at least an hour after the giving of the anesthetic and shortly before the beginning of the high pressure exposure. This first sample is considered the normal or control sample for

* This study was aided in part by the Miriam Smith Rand Fund of Harvard University.

each experiment. Blood samples during the oxygen pressure exposure were usually taken every 15 minutes for the first hour and every 30 minutes for the remainder of the exposure. This was varied occasionally in an attempt to get samples immediately before, during, and after any convulsive seizure. The number of samples taken was never less than 3 and was usually 8. Arterial blood was taken from the femoral artery, and mixed venous blood from the right side of the heart, but as no chemical difference was noted they will be considered as identical.

The blood for calcium and potassium was put directly into centrifuge tubes and allowed to clot, and then was sent out of the compression chamber. The blood for the remainder of the determinations was immediately put into beakers containing oxalate and was stirred. Because of the bubbling and hemolysis which take place in blood drawn at a high pressure and then reduced to atmospheric pressure, it was found absolutely necessary to measure or pipette the blood while under the pressure at which it was taken in order to obtain accurate results. For each sample taken, the oxalated blood for phosphorus determination was measured directly into the trichloroacetic acid and sent out of the compression chamber to be filtered. The oxalated blood for the other determinations was measured into flasks and the distilled water added, and it was then sent out for the further steps of protein precipitation.

METHODS OF ANALYSIS. The methods used for the various determinations were as follows:

The removal of the protein was accomplished by the use of sodium tungstate and sulfuric acid (Folin and Wu, 1919). This protein-free filtrate was used to determine sugar, non-protein nitrogen, chlorides, creatinin, and lactic acid.

Blood sugar was determined by the method of Folin and Wu (1929).

Non-protein nitrogen was determined by the method of Folin (1927a).

Chloride content was determined by the method of McLean and Van Slyke (1915) and expressed as sodium chloride.

Preformed creatinin was determined by the method of Folin (1927b).

Inorganic phosphate was determined on the whole blood by the method of Fisk and Subbarow (1925), and is reported as milligrams of phosphorus per 100 cc. of blood.

Calcium was determined on serum by the method of Fiske and Logan (1931).

Potassium was determined on serum by the method of Breh and Gaebler (1930).

EXPERIMENTAL RESULTS. *Control animals.* The effect of the anesthetic, sodium barbital, was determined upon 3 dogs breathing room air over a period of time similar to the time of the experiments. These animals had nothing done to them other than the minor operative procedure

necessary for the taking of the blood samples. The chemical changes were negligible, as will be noted by reference to table 1, which records the findings of a typical control. Our normal findings check with the work of Arnold and Mendel (1927) who used a similar anesthetic; and with the normal blood chemistry of the dog as reported by Dill, Edwards, Florkin and Campbell (1932). The control sample (no. 1) for the other 17 dogs was within the same range as the 3 controls, and thus we have further evidence of the steady condition existing under sodium barbital anesthesia.

Experimental animals. The blood sugar was determined in 17 experiments. In 10 of these no significant change from the normal values was found, 1 showed a slight decrease, and 6 showed a significant increase. Of

TABLE 1

Constancy of the chemical composition of the blood in dogs anesthetized with sodium barbital and breathing room air. Control no. 1-B. Weight, 18.5 kilos

SAMPLE NUMBER	TIME OF TAKING SAMPLE	MGM. PER 100 CC. BLOOD						
		Sugar	Phosphorus	N.P.N.	Chlorides	Potassium	Calcium	Creatinin
	9:37 a.m.	Sodium barbital intraperitoneally						
1	10:56	114	6 9	41 7	570	13 0	12 8	1 5
2	11:40	111						
3	12:05 p.m.	110						
4	12:15	114						
5	12:25	116	7 1	43 8	564	12 5	11 4	1 5
6	12:55	114						
7	1:55	114	7 6	40 8	560	13 0	10 4	1 5
8	4:15	110	7 6	40 8	552	12 5	12 2	1 3
	4:20	Hemorrhage of 220 cc. making total of 380 cc. lost*						
9	4:30	111	7 2	43 8	554	13 0	10 8	1 5

* Hemorrhage does not appear to be a factor in changing the blood chemical findings.

these 6, 2 had severe convulsions with recovery, 1 had convulsions leading to death during the experiment, 1 had convulsions and a period of apnea followed by death during the experiment, 1 had convulsions and a period of apnea with recovery, and 1 had a period of apnea with no convulsions and was bled to death. Of the 10 dogs showing no marked change in sugar and the one with slight sugar decrease, not one showed any of the above symptoms. Thus, there is a clear association between the extreme manifestations of oxygen poisoning and the sugar increases noted.

Phosphorus was determined in 8 experiments with no change from the normal in 5 and a marked change in 3. Of the 3 showing a change 1 had marked convulsions leading to death, 1 had convulsions, apnea and died, and 1 had convulsions with recovery. The 5 showing no change showed

none of the above symptoms. Again, as with the sugar, significant increases occurred when oxygen poisoning was extreme.

Non-protein nitrogen showed no change in 8 and slight increase in 5 of the 13 experiments in which it was determined. Of the 8 showing no change, 2 had severe convulsions leading to death; of the 5 showing increase, 1 had apnea and convulsions, 1 convulsions, and 1 apnea alone. It is evident that the slight increases noted in the non-protein nitrogen have no relation to oxygen poisoning.

Chloride content was determined in 13 experiments, with no change being noted in 3, increases in 4 and a decrease in 6. No apparent relation to convulsions or other symptoms was noted.

Creatinin showed no change in the 4 experiments in which it was determined.

Calcium was determined in 10 experiments, with no change being noted in 8, increase in 1 and decrease in 1. There was no relation to oxygen poisoning.

Potassium was determined in 10 experiments, and showed no change in 4, increase in 3, and decrease in 3. No relation to oxygen poisoning was noted.

Since there seemed to be an association between severe manifestations of oxygen poisoning and certain of the chemical findings, it was desirable in order to compare the findings to divide the animals into the following groups: 1, those having no marked disturbance during exposure; 2, those animals having only convulsions followed by recovery, and 3, those animals having long periods of apnea, severe convulsions, or death during exposure.

The type of convulsion noted consists of a violent spasm of the head, neck, chest, and forelegs accompanied by a vigorous shaking movement and a rapid inspiration. These spasms occur from 1 to 15 times a minute and last from 1 to 3 seconds. They terminate abruptly with relaxation and expiration. Periods of apnea may occur between spasms.

Group 1. Animals having no marked symptoms. Eleven animals fall into this group which showed no convulsions, no apnea, and did not die during exposure; and as a group there were very slight changes in the chemical findings. Table 2 gives the findings in 2 typical experiments of this group. Any doubt as to the effect of the anesthetic over a long period of time is dispelled by experiment 25-B in which the last sample, taken 10 hours and 18 minutes after the anesthetic was given, was substantially the same as the first sample taken 1 hour and 33 minutes after the anesthetic was given. It is even more remarkable that in this dog an exposure to 97 per cent oxygen at a pressure of 45 pounds gauge, or in effect 388 per cent oxygen, for a period of 4 hours and 7 minutes caused no noteworthy change in any of the blood chemical constituents studied. In

experiment 15-C the findings were also remarkably constant during a similar exposure lasting 2 hours. None of the other 9 dogs in this group showed any greater variation in any of the blood constituents than did the control dogs. From this group, one is forced to conclude that high tension oxygen of itself causes no significant chemical changes in the blood of dogs exposed for periods of from 2 to 4 hours.

TABLE 2

Constancy of the chemical composition of the blood in dogs anesthetized with sodium barbital and exposed to a high tension of oxygen but showing no symptoms of poisoning

SAMPLE NUMBER	TIME OF TAKING SAMPLE	MGM. PER 100 CC. BLOOD						
		Sugar	Phosphorus	N.P.N.	Chlorides	Potassium	Calcium	Creatinin
Experiment no. 25-B. Weight of dog, 26 kilos								
	9:07 a.m.	Sodium barbital intraperitoneally						
1	10:40	116	3.8	35.1	560	10.7	11.9	1.4
	12:45 p.m.	45 pounds oxygen pressure						
2	1:00	112						
3	1:22	109	5.3	34.2	548	11.6	11.1	1.5
4	2:22	109						
5	3:30	99	4.2	35.4	538	13.2	11.0	1.4
6	4:30	109						
	4:52	Decompression of 142 minutes by standard stages—Off O_2						
7	7:25	105	3.6	Lost	520	12.7	10.5	1.5
Experiment no. 15-C. Weight of dog, 19.5 kilos								
	9:40 a.m.	Sodium barbital intraperitoneally						
1	11:15	107	3.1	47.1	592	10.4	Lost	1.4
	12:35 p.m.	45 pounds oxygen pressure						
2	12:45	111	3.5					
3	12:57	105	3.4	46.8	592	10.4	10.9	1.4
4	1:14	103	3.3					
5	1:30	96	3.5	47.4	592	10.1	11.2	1.4
6	1:50	96	3.6					
7	2:10	91	3.7	53.4	574	10.2	10.8	1.5
8	2:28	94	3.4					
	2:32	Decompression						

Group 2. Animals having convulsions followed by recovery. There were two in this class. In experiment 13-C, the sugar taken every 30 minutes during exposure increased as follows: 124, 138, 142, 140, and 168 mgm. per 100 cc. of blood. Phosphorus showed a slight increase also. The other experiment in which mild convulsions occurred was a 60-pound exposure. The sugar readings taken every 15 minutes during the exposure

were: 132, 120, 114, lost, 106, 110, 123, and 140 mgm. per 100 cc. In this experiment the other constituents, all of which were studied, remained even more constant than the sugar. It is perfectly evident that neither of these experiments showed changes of sufficient magnitude to be considered significant in relation to convulsions.

Group 3. There were 4 animals which showed severe manifestations of oxygen poisoning. Two of these dogs had long periods of apnea which without doubt had an effect on the blood composition because of the associated increase of carbon dioxide. (Asphyxia, as it is commonly considered, does not exist under these conditions because the venous blood is found to be saturated with oxygen.) In experiment 16-B the animal became apneic after 75 minutes' exposure to high tension oxygen and continued so until bled to death 70 minutes later. The sugar content of the normal blood sample taken 1 hour after the anesthetic was given was 172 mgm. per 100 cc. The blood taken 80 minutes after the beginning of oxygen exposure and after 10 minutes of apnea contained 198 mgm., and after 140 minutes of exposure and 70 minutes of apnea contained 204 mgm. of sugar per 100 cc. Phosphorus was not estimated and the other constituents showed no significant change. In experiment 18-B the dog had a 56 minute period of apnea starting after 60 minutes' exposure to high tension oxygen. This apnea was broken by convulsive gasps or convulsive respiration at frequently recurring intervals. Normal respiration returned during decompression 6 minutes after removal of oxygen. The sugar content of the normal sample taken 1 hour and 35 minutes after the anesthetic was given was 129 mgm. per 100 cc. The blood taken 65 minutes after the beginning of the high tension oxygen exposure and 5 minutes after the beginning of apnea contained 140 mgm., and after 2 hours and 10 minutes' exposure and 1 hour and 15 minutes of intermittent convulsion and apnea it contained 185 mgm. of sugar per 100 cc. Phosphorus was not estimated and the other constituents showed no change. The other 2 dogs (24-B and 26-B) died during the exposure to high tension oxygen. Both of these experienced severe convulsions and 24-B had a period of apnea before death. These two experiments are reported in table 3, and again there are changes in sugar and phosphorus of significant magnitude. In these two experiments lactic acid increases were also significant.

In general urine examinations were not significant. Several dogs showed small amounts of albumen and most of them showed some sugar at the end of the experiment. The urine of almost all had a high specific gravity. None showed acetone. Cisternal fluid, examined in 5 dogs, was clear in all cases. The cell count was not increased. Protein in the fluid was negative by Lay's reaction. The sugar was increased in all of the 5 animals in which it was studied but it was taken at the very end of the experiment and cannot be said to be significant.

DISCUSSION. In considering the results, the most striking finding is the constancy of the chemical constituents of the blood even under very severe experimental conditions. That anesthetized dogs can breathe approximately 400 per cent oxygen for 4 hours without any change even in the blood sugar seems remarkable.

TABLE 3

Changes in the chemical composition of the blood of dogs anesthetized with sodium barbital, exposed to a high tension of oxygen and showing symptoms of severe oxygen poisoning

SAMPLE NUMBER	TIME OF TAKING SAMPLE	MGM. PER 100 CC. BLOOD						
		Sugar	Phosphorus	N.P.N.	Chlorides	Potassium	Calcium	Creatinin
Experiment no. 24-B. Weight of dog, 17.3 kilos								
	9:05 a.m.	Sodium barbital intraperitoneally						
1	10:40	108	4.7	34.5	530	17.0	13.2	1.4
	11:10	Oxygen at atmospheric pressure—100 per cent						
2	11:50	126						
	12:03 p.m.	45 pounds oxygen pressure						
3	12:13	133						
4	12:23	133						
5	12:33	127	6.1	39.9	534	15.3	14.3	1.4
	1:16	Violent convulsions start. Recurrent attacks						
	1:50	Apnea between attacks						
6	2:20	180						
	2:35	Death						
Experiment no. 26-B. Weight of dog, 24.5 kilos								
	9:00 a.m.	Sodium barbital intraperitoneally						
1	10:00	117	4.8	38.7	534	11.0	13.0	1.5
	11:58	45 pounds oxygen pressure						
2	12:15 p.m.	144						
3	12:34	141						
4	12:55	139	5.1	41.4	510	10.7	12.8	1.5
	1:35	Marked convulsions start. Recurrent attacks						
5	1:45	136		39.9	498			Lost
6	2:00	141						
7	2:25	250	7.1			Lost	12.7	
	2:30	Death						

It is logical to assume that, if there is no abnormal chemical change from breathing 400 per cent oxygen for 4 hours, there would be none from breathing lower oxygen tensions. Since the physiological effects of compressed air are considered to be due to the partial pressure of the oxygen it may be held that there are no abnormal chemical changes in the blood of men

working in compressed air at any of the pressures now used in caisson or tunnel construction, for, even at 5 atmospheres absolute or 60 pounds gauge the oxygen tensions would be only 100 per cent and two-thirds of our animals tolerated 400 per cent of oxygen.

SUMMARY

Twenty dogs were used in a study of the blood chemistry changes produced by breathing oxygen at high tensions.

The animals having convulsions, or apnea, or those dying as a result of the exposure showed changes in the sugar and phosphorus content of the blood. These increases in sugar and phosphorus were considered to be the result of the toxic effect of increased oxygen tensions but not to be an etiological factor in either the convulsions or the lung damage.

In view of these findings, it seems very improbable that men working under air pressure of 5 or 6 atmospheres would be subject to oxygen tensions which could in any way affect the chemical constituents of the blood.

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ACIDOSIS: ACID INTOXICATION, OR ACARBIA?[1]

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The discovery that monoiodoacetic acid has the power to prevent the formation of lactic acid in the living body (1) makes possible a crucial experiment as to the real nature of the state now called acidosis.

This state is now generally regarded as an acid intoxication. It is believed to be essentially similar to that induced experimentally by the ingestion or intravenous injection of hydrochloric acid. Acids are supposed to be liberated in the body under oxygen deficiency from any cause: intense muscular exertion, exposure to great altitudes, carbon monoxide, fever especially in children, alcoholic intoxication, ether anesthesia, physical injuries and crushing of tissues, deficiency of insulin, as well as by an unbalanced diet.

The symptoms in all such cases are now commonly assigned in large part to acidosis in the sense of acid intoxication. The acid most readily produced in the body is lactic acid. If then lactic acid is not a cause of acid intoxication, it is doubtful whether any acid produced in the body can intoxicate. All such acids would then have to be regarded as mere accessories of the conditions they are now supposed to induce. The conditions called acidosis would not be acid intoxication.

The outstanding phenomena of acidosis are the decrease of the bicarbonates of the blood and the increase of breathing. Walter (2) noted these symptoms in his classic observations on animals to which he administered hydrochloric acid. J. B. S. Haldane (3) observed them in himself after ingestion of ammonium chloride. Stadelmann (4) is generally regarded as the originator of the acidosis theory. He first pointed out that in diabetic acidosis symptoms occur closely similar to those of experimental acid intoxication. Araki (5) found an increased production of lactic acid in carbon monoxide asphyxia.

L. J. Henderson (6) applied the modern chemical conception of ionic equilibrium and H-ion concentration to the blood and recast the conception of acidosis as a disturbance of the acid-base equilibrium of the blood. Van

[1] The experimental data upon which this paper is based are contained in a dissertation submitted by the junior author to the Graduate School of Yale University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1933.

Slyke (7) emphasized the importance of the bicarbonates and developed the idea that they are the "alkaline reserve" that protects the blood from acidity. Many investigators have noted the symptoms of acidosis as the result of deficiency of oxygen. Recently Eastman (8) has reported finding a decrease of bicarbonates, low pH, and increase of lactic acid in the blood of stillborn babies: a typical acute asphyxial acidosis.

From these conceptions and a vast number of experimental and clinical observations two particularly important inferences have been drawn in regard to so-called acidosis. One is that in this state a low content of bicarbonates in the blood is necessarily due to neutralization of the alkali by acid. The other is that a low pH in the blood indicates an excess of acids. Both of these conceptions are almost purely chemical. Neither takes account of the self-regulating capacity of the living system or stresses the fact that the constancy of the composition of the circulating blood is maintained by a continual physiological regulation.

It is known, for example, that a marked decrease of the bicarbonates of the blood may occur without the loss of alkali from the body, or the intake or production of acid (9). Mere overventilation of the blood during its passage through the lungs is often sufficient to induce this compensatory effect. Contrariwise an increase of the carbon dioxide pressure in the blood under the influence of respiratory depression, as by morphine, may call an extra amount of bicarbonates into use in the blood. These are reactions of the widest biological significance.

Even in plants, as Thornton (10) reports, an increased pressure of carbon dioxide in the atmosphere induces an increase, not of acidity, but of alkalinity and of the bicarbonates in the tissue juices. That this is a vital reaction, and not a mere chemical effect, is demonstrated by the fact that in plants as in animals the transfer of alkali from tissues to fluids occurs only in the presence of oxygen. In the absence of oxygen carbon dioxide causes a change of pH, not toward alkalinity, but acidity. In this, as in many other vital functions, chemical forces act in one direction; but the biological system reacts more strongly in the contrary direction.

There is as yet no term to express the condition of low blood bicarbonates, when not produced through the acidotic process. We suggest for this condition the term "acarbia."

As regards the pH of the blood, an especially erroneous inference from chemical theory is now common. The H-ion concentration of the blood is not an expression of the balance of the fixed and non-volatile acids against the bases. On the contrary the pH of the blood is simply, and almost solely, an expression of the relation of the carbon dioxide in solution—or carbonic acid—to the bicarbonates. As the amount of carbon dioxide in simple solution is absolutely controlled by respiration, what the pH of the blood really tells is whether the sensitivity of the respiratory center and its

afferent connections is normal, or above or below normal. Respiration is capable of a tenfold, or 1000 per cent, variation. A mere doubling, or 100 per cent increase, of pulmonary ventilation is a small, easy, and scarcely noticeable alteration when induced by interior conditions. It involves no more than the difference in respiration when one is sitting still or walking at two miles per hour. Such an increase of breathing in a quiescent acidotic patient would lift a pH as low as 7.1 back to a normal value of 7.4. If respiration were reacting normally, it would make that increase and restore the normal pH; but in acidosis, in the sense of low pH, the low sensitivity holds the pH at the acidotic level.

The only immediate cause of acidosis, in the sense of low pH, is therefore an inadequate activity on the part of respiration. The problem lies, not in the balance of acids and bases, but rather in the conditions or substances that increase and decrease the sensitivity of the respiratory center. Among these conditions, apart from drugs and disease, the best recognized is anoxemia.

In the search for substances that influence the sensitivity of the respiratory center to its specific stimulus, the pH of the blood, investigators (11) have again and again suggested that lactic acid must be one of the important respiratory stimulants. This suggestion has applied particularly to the so-called acidosis of asphyxia and to conditions, such as anesthesia, related to asphyxia. Whether or not lactic acid has this significance and importance is however open to considerable doubt. There are in fact numerous substances—non-acids, such as acetone, ethyl ether, sulphides, and various drugs, such as lobelin, methylene blue and monoiodoacetic acid, and many tissue extracts—that exert marked effects upon respiratory sensitivity when introduced into the blood. The importance heretofore assigned to lactic acid in many physiological relations is now questioned by an increasing number of investigators (12).

Fortunately the part played by lactic acid in asphyxial acidosis can now be tested by means of monoiodoacetic acid. It is administered, of course, as a neutral salt. If decrease of bicarbonates under anoxemia is due to neutralization by lactic acid, there should be no decrease of bicarbonates under anoxemia, when the formation of lactic acid is prevented. If lactic acid either summates its acid property with carbonic acid or otherwise sensitizes respiration, there should be no hyperpnea in an animal treated with monoiodoacetic acid and then subjected to deficiency of oxygen.

As a matter of fact, as the results to be here reported show, the effects of anoxemia upon the bicarbonates of the blood and upon respiration are essentially the same in animals that have received monoiodoacetic acid as in those that have not. This fact shows clearly that the production of lactic acid under anoxia is not an essential element in the decrease of the bicarbonates and increase of respiration. The only difference observable

in the two groups of animals is that those iodized do not survive to as low an oxygen in the inspired air and as low a pH in the blood under asphyxia, as do the controls.

These observations indicate that the increase of lactic acid and lowering of pH in acute asphyxia are not toxic. This acidosis tends rather to save life.

EXPERIMENTAL PROCEDURES. The technique of our experiments was essentially the same as that in the recent investigation along similar lines by Henderson and Radloff (13). Dogs were made to rebreathe air in a closed system through a cartridge of sodium hydroxide. Respiration was recorded quantitatively by the movement of a small spirometer in the system. The animals were only lightly morphinized: 0.01 gram of morphine sulphate per kilo body weight. The trachea and femoral or carotid artery were exposed and cannulated under novocaine. Blood samples were drawn under oil in test tubes lightly coated with sodium oxalate and sodium fluoride, and were kept on ice until the time for equilibration and analysis. The carbon dioxide content and capacity were determined with the manometric apparatus of Van Slyke and Neill; lactic acid both by the methods of Friedemann, Cotonio and Shaffer (14) and by that of Bruno Mendel (15). The pH of the serum was determined by the quinhydrone electrode of Cullen (16), that of the whole blood by the hydrogen electrode of DuBois(17). The monoiodoacetic acid, neutralized with sodium bicarbonate, was always injected intravenously in a 1 per cent solution during 10 minutes. The volume of air breathed per minute was determined by measuring and counting the graphic record of the rise and fall of a small spirometer attached to the rebreathing apparatus.

Control experiments without monoiodoacetic acid. These experiments gave results closely similar to those of Henderson and Radloff. Those authors were chiefly interested in the effects of anoxemia and asphyxia upon respiration. Our attention has been chiefly directed to the changes in the chemical condition of the blood. The results from experiments on eight dogs are given in table 1.

From the data of the first three analyses in each experiment it appears that in these normal animals under progressive decrease of oxygen in the inspired air from 20.9 down to 7 per cent of an atmosphere, there was a more than threefold increase in respiration, inducing a distinct rise of pH and a decrease of the bicarbonates in the blood from a mean of 42 volumes per cent to a mean of 36.4. The lactic acid, on the contrary, was not appreciably increased, but remained practically unchanged. The mean value when the animals inspired room air was 33 mgm. per cent. The value under 7.0 per cent of oxygen was only 35 mgm. per cent.

The fourth group of analyses in the table shows the conditions when the oxygen was reduced to a fatally asphyxiant degree. Then, and then only,

TABLE 1

Showing the changes in respiration and blood under progressive anoxemia

DOG	TIME	O_2 OF INSPIRED AIR	RESPIRATION	CO_2 CONTENT	CO_2 CAPACITY	pH OF SERUM	pH OF BLOOD	LACTIC ACID
	minutes	*per cent*	*liters per min.*	*vol. per cent*	*vol. per cent*			*mgm. per cent*
First blood sample (Normal)								
A (18 kilo)	0	20 0	--	48 0	38 5	7 39	—	30
B (15 kilo)	0	20 5	—	42 0	41 8	7 42	—	18
C (20 kilo)	0	20 9	3 0	41 0	39 0	7 38	—	47
D (15 kilo)	0	20 9	2 0	38 0	49 0	7 44	—	20
E (12 kilo)	0	20 9	2 0	50 5	40 2	7 41	—	50
F (10 kilo)	0	20 2	2 0	40 5	43 7	7 39	—	35
Average	0	20 6	2 3	43 3	42 0	7 40	7 27*	33
G (14 kilo)	0	20 9	—	28 4	32 0	7 39	7 22	—
H (10 kilo)	0	20 9	—	39 4	40 5	7 41	7 28	—
Second blood sample (Slight anoxemia)								
B	20	17 7	—	38 2	40 0	7 44	—	30
C	15	18 3	3 2	37 5	35 3	7 47	—	38
F	15	18 0	2 5	36 3	40 2	7 44	—	35
Average	17	18 0	2 9	37 3	38 5	7 45	7 48*	34
Third blood sample (Increasing anoxemia)								
A	40	6 8	—	40 8	30 2	7 48	—	30
B	50	7 5	—	32 5	35 3	7 48	—	35
C	55	7 0	8 0	23 0	25 2	7 48	—	40
D	45	8 2	11 0	34 0	57 0	7 57	—	22
E	50	6 0	10 0	40 2	35 5	7 44	—	41
F	45	7 0	6 5	28 0	35 5	7 45	—	42
Average	47	7 1	8 8	33 1	36 4	7 48	7 56*	35
Fourth blood sample (Asphyxia)								
A	50	4 0	—	22 3	21 9	7 35	—	162
B	70	4 0	—	20 8	25 0	7 38	—	129
C	65	4 5	2 0	18 4	14 5	7 29	—	135
D	55	4 5	2 7	22 0	37 0	7 42	—	63
E	60	4 0	3 0	19 0	20 5	7 35	—	150
F	55	3 5	2 7	15 2	19 4	7 35	—	109
Average	59	4 1	2 6	19 6	23 0	7 36	7 10*	125
G	76	6 0	—	22 0	24 5	7 38	7 17	—
H	78	5 5	—	19 7	16 8	7 37	7 15	—

Dogs A, B, C, D, E and F died immediately after 4th blood sample was taken.

Dogs G and H were allowed to recover from an almost fatal asphyxia.

The figures indicated with an asterisk are not direct analyses by means of the glass electrode, as the other figures in the same column are, but are obtained by means of the line in figure 1 from the determinations with quinhydrone

TABLE 2

Showing the influence of carbon dioxide in recalling alkali to the blood and hastening recovery after asphyxia

DOG	TIME OF ASPHYXIA	INSPIRED AIR	O_2 CONTENT	CO_2 CAPACITY	pH OF SERUM	pH OF BLOOD	LACTIC ACID
	minutes		*vol. per cent*	*vol. per cent*			*mgm. per cent*
Initial blood samples (Normal)							
Q	0	Air	40 0	41 0	7 41	7 30	30
R	0	Air	41 0	40 0	7 40	7 26	20
G	0	Air	28 4	32 0	7 39	7 22	—
H	0	Air	39 4	40 5	7 41	7 28	—
S	0	Air	40 0	43 0	—	—	—
T	0	Air	39 0	41 0	—	—	25
Second blood samples (Asphyxia)		*per cent oxygen*					
Q	45	3 0	23 0	24 0	7 38	7 16	62
R	45	4 0	13 0	22 0	7 36	7 09	100
G	45	6 0	22 0	24 5	7 38	7 17	—
H	45	5 5	19 7	16 8	7 38	7 15	—
S	45	3 5	11 0	26 0	—	—	—
T	45	4 0	10 0	28 0	—	—	70

Blood samples during recovery, dogs Q and R breathing air, dogs G, H, S and T breathing air + 8% carbon dioxide

	TIME OF RECOVERY IN MINUTES						
Q	20	Air	31 0	33 0	7 39	7 21	50
	55	Air	40 0	40 0	7 40	7 26	35
R	8	Air	16 0	24 0	7 37	7 13	—
	60	Air	37 0	36 0	7 39	7 21	30
G	12	Air + 8% CO_2	31 1	32 0	7 36	7 09	—
H	12	Air + 8% CO_2	45 9	38 0	7 30	6 95	—
S	8	Air + 8% CO_2	30 0	28 0	—	—	—
	15	Air	20 0	38 0	—	—	—
T	8	Air + 8% CO_2	28 0	31 0	—	—	50
	15	Air	20 0	39 0	—	—	40

was there a marked increase of lactic acid: an increase up to an average of nearly four times the normal amount. Simultaneously respiration, instead of increasing, was depressed. Shortly after the last blood samples were taken, six of the animals died of asphyxia. Two were restored with fresh air. The oxygen in the inspired air had been reduced to levels between 3.5 and 4.5 per cent.

These observations, in agreement with those of Henderson and Radloff, demonstrate that the increase of respiration and the decrease of bicarbonates under a diminished supply of oxygen, short of a fatally asphyxiant level and above 7 per cent, are not to any appreciable degree due to formation of lactic acid and neutralization of bicarbonates by that acid.

Toxicity of monoiodoacetic acid. Before using monoiodoacetic acid for the primary purpose of this paper—inhibition of lactic acid formation—we determined the dose that would be small enough not to kill or seriously disturb the functional normality of a dog; and yet that would be large enough to prevent the formation of lactic acid even under fatal asphyxia. Even with 40 mgm. the result was fatal in all three of the experiments in which this dosage was used. On the other hand, with a dosage of 25 mgm. per kilo, a considerable formation of lactic acid occurred, when the animals were subjected to a progressive deprivation of oxygen to the point of fatal asphyxia. The dosage finally adopted was 33 mgm. per kilo body weight. In two animals death occurred soon after this amount was administered.

In five experiments, in which the animals received 33 mgm. per kilo and recovered from the immediate effects, the results shown in the first half of table 3 were obtained. In all of these experiments the injection of monoiodoacetic acid was followed in the course of two or three minutes by a progressively increasing volume of breathing up to two or three times the normal. As a compensation for the resulting acapnia, the bicarbonates of the blood underwent a considerable decrease. During this stage oxygen was added to the closed system, into which the animals breathed, so as to maintain the percentage of oxygen at or slightly above that in room air.

After about three-quarters of an hour the condition of the animals returned nearly to normal as regards respiration and bicarbonates. The following experiments on asphyxia were then performed.

Changes in the blood under progressively diminishing oxygen in dogs incapable of producing lactic acid. As soon as the iodized animals had recovered from the respiratory effects of monoiodoacetic acid the rebreathing system was flushed with room air. Rebreathing was then started and continued until death. The analytical results obtained are shown in the second half of table 3.

As these data indicate, the animals were now incapable of producing an increase of lactic acid; at least, none occurred in any case. But this incapacity did not prevent an increase of respiration and a progressive

decrease of bicarbonates of exactly the same type and of virtually the same amount as in the control experiments summarized in table 1.

TABLE 3

Showing the changes in respiration and blood in dogs treated with monoiodoacetic acid, and then—after recovery from the respiratory effects of the drug—asphyxiated

DOG	TIME	O_2 OF INSPIRED AIR	RESPIRATION	CO_2 CONTENT	CO_2 CAPACITY	pH OF SERUM	pH OF BLOOD	LACTIC ACID
	minutes	*per cent*	*liters per min.*	*vol. per cent*	*vol. per cent*			*mgm. per cent*
First blood sample (Normal)								
I (13 kilo)	0	25 0	3 0	43 2	48 5	7 37	—	35
J (12 kilo)	0	25 0	3 5	41 6	45 0	7 39	—	14
K (11 kilo)	0	30 0	3 8	42 3	45 5	7 42	—	37
L (14 kilo)	0	25 0	3 8	36 8	40 0	7 40	—	40
M (9 kilo)	0	25 0	1 9	40 0	44 2	7 42	—	28
Average	0		3 3	40 8	44 6	7 40	7 26*	31
N (7 kilo)	0	20 9	—	38 8	40 5	7 39	7 20	35
O (11 kilo)	0	20 9	—	43 3	39 5	7 40	7 27	45
P (12.5 kilo)	0	20 9	—	40 9	35 0	7 39	7 20	40
Second blood sample (Hyperpnea after injection of monoiodoacetate)								
I	10	25 0	16 0	26 8	39 0	7 43	—	38
J	15	25 0	7 8	33 2	36 5	7 44	—	15
K	15	30 0	10 3	26 9	36 0	7 48	—	41
L	10	25 0	15 5	20 5	32 1	7 45	—	45
M	10	25 0	5 5	29 8	35 5	7 48	—	28
Average	12	—	11 0	27 4	35 8	7 45	7 48*	33
Third blood sample (Return of normal breathing)								
I	35	25 0	3 8	40 0	47 6	7 39	—	36
J	45	25 0	3 4	39 7	40 9	7 38	—	17
K	50	30 0	3 5	38 9	42 7	7 41	—	39
L	40	25 0	3 5	35 3	38 9	7 41	—	39
M	40	25 0	2 3	38 3	39 7	7 40	—	30
Average	42	—	3 3	38 4	41 9	7 39	7 24*	32
O	42	20 9	—	36 5	39 2	—	7 26	39
Fourth blood sample (Slight anoxemia)								
I	52	18 5	4 0	35 4	45 0	7 40	—	34
J	57	18 5	4 5	36 0	36 8	7 40	—	18
K	54	18 2	4 0	35 2	40 0	7 41	—	38
L	57	18 1	4 0	32 8	37 0	7 42	—	40
M	77	18 6	2 8	35 0	36 0	7 41	—	30
Average	55	18 4	3 9	34 9	38 9	7 41	7 30*	32
Fifth blood sample (Increasing anoxemia)								
I	82	8 0	10 0	23 0	35 2	7 40	—	34
J	87	8 0	14 8	25 8	28 5	7 41	—	18
K	82	8 3	9 0	23 5	30 0	7 44	—	36
L	92	8 7	8 0	21 5	30 3	7 48	—	38
M	82	7 1	6 8	24 8	27 1	7 43	—	33
Average	85	8 0	9 7	23 7	30 2	7 43	7 40*	32
N	72	8 5	—	25 9	33 8	7 42	7 36	32
O	69	10 0	—	25 9	31 8	7 43	7 41	40
P	77	9 5	—	24 2	31 8	7 45	7 51	41
Sixth blood sample (At the point of death from asphyxia)								
I	92	6 0	5 5	19 1	27 0	7 38	—	34
J	92	6 0	4 0	17 3	20 0	7 37	—	16
K	87	6 5	1 8	19 0	22 3	7 39	—	36
L	97	5 0	4 0	18 0	24 6	7 39	—	39
M	92	5 5	1 7	18 0	20 0	7 39	—	31
Average	92	5 8	3 4	18 3	22 8	7 38	7 19*	31
N	79	7 0	—	24 0	26 9	7 41	7 31	33
O	77	7 5	—	22 3	27 5	7 47	7 55	37
P	88	7 5	—	33 4	29 4	7 44	7 45	38

*The figures indicated with an asterisk are not direct analyses by means of the glass electrode, as the other figures in the same column are, but are obtained by means of the line in figure 1 from the determinations with quinhydrone.

The observations on dogs I to M were made in the winter. Those on N, O, and P were made in very hot weather; and the final pH values are considerably affected by heat polypnea.

The iodized animals died at 5 to 6.5 per cent oxygen in the inspired air; the undrugged controls at the distinctly lower figures 3.5 to 4.5. But even this difference appears to weigh against the idea of a "lactic acid intoxica-

tion" as an injurious factor in asphyxial acidosis; for in three experiments (not shown in the tables) in which iodized animals were asphyxiated, respiration and life were prolonged down to 4 per cent of oxygen when (racemic) lactic acid was injected into a vein.

Variations of the pH of the blood and serum during the development of asphyxia. In all of the experiments here reported the pH of the serum of the blood was determined with the quinhydrone electrode. In a number of the experiments (G, H, N, O, P, I, and R) the pH of the whole blood was also determined by means of the glass electrode. For the latter determinations we are indebted to our colleague, Mr. Delafield DuBois; and we take this opportunity to express our appreciation and thanks.

As all of the animals had received morphine, some degree of lowering of pH is to be expected in the first or "normal" samples. This lowering is shown by the glass electrode, but not by quinhydrone. The much greater lowering of pH under acute asphyxia is shown by the determinations with the glass electrode on whole blood, but not to a correct extent by the determinations with quinhydrone on serum. The quinhydrone electrode generally gives fairly accurate figures within the normal variations of pH and shows the direction of abnormal variations, but fails seriously to indicate their full extent. This is particularly true in regard to the lowering of pH under acute asphyxia.

Although the figures obtained by the two methods are often not the same, their relations are so definite that we have constructed figure 1 to express them. From the line in this figure the values obtained by one method may be transposed into the values obtainable by the other. We have therefore felt justified in placing in tables 1 and 2 the values for the average pH of the experiments A to F and I to M in terms of the glass electrode, although in fact all of the determinations summarized in the averages in these experiments were actually made only by means of quinhydrone. Determinations of pH by both methods were made in experiments G, H, N, O, P, Q, and R.

It should also be stated that while the experiments on dogs A to F and I to M were made during the winter months, those on G, H, N, O, P, Q, and R were made during extremely hot summer weather. The latter group, especially N, O, and P, were distorted by heat polypnea. They died with a pH far higher than the iodized dogs observed in the winter.

Recovery from asphyxial acidosis hastened by further acidifying the blood by inhalation of carbon dioxide. If asphyxial acidosis involves an acid intoxication, inhalation of carbon dioxide should be harmful. If on the contrary a further lowering of pH by inhalation of carbon dioxide tends to recall alkali to the blood, such an inhalation should speed recovery from asphyxia. A vast experience shows that in the treatment of human cases of asphyxia carbon dioxide is as important for rapid resuscitation as a renewed supply of oxygen (18).

In table 2 are given the results on two dogs that after asphyxiation were left to recover in room air, and on four treated with inhalation of 8 per cent carbon dioxide in air. The results show a much more rapid return of the blood alkali in the latter cases than in the former. In spite of the extreme depression of pH—in one case below 7.00—the inhalation of carbon dioxide exerted an essentially remedial influence.

In cases of asphyxia of newborn babies Eastman (8) has found the pH of the blood as low as 7.00. He infers that to force the pH to an even lower figure by inhalation of carbon dioxide must "intensify acidosis" and be

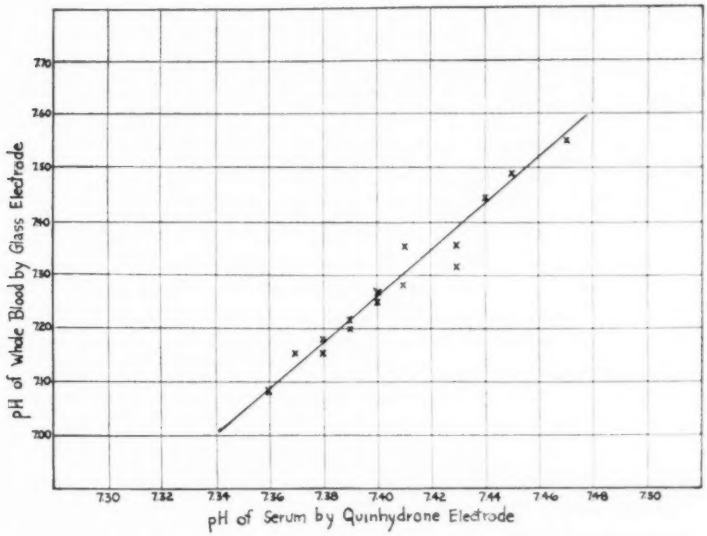

Fig. 1. Showing the relations of the figures for the pH of the serum obtained with quinhydrone and those of the whole blood with the glass electrode.

harmful. Yet clinical experience shows that the lives of many babies are now being saved by inhalation of carbon dioxide in spite of the "intensification of acidosis." For deeply asphyxiated babies, not merely 5 or 8 per cent of carbon dioxide is used, and needed, to save life. In extreme cases 20 per cent carbon dioxide is used successfully, when no weaker stimulus is effective, although the temporary acidosis must be intense.

Relations of oxygen and carbon dioxide in the control of respiration during the development of asphyxia and during recovery. Heymans and his collaborators (19) have demonstrated that the sudden acute oxygen deficiency induced by inhalation of nitrogen acts through the sinus caroticus, rather than directly upon the respiratory center. This observation has been con-

firmed by Gemmill and Reeves (20), who regard it as a crucial refutation of the theory that "lack of oxygen increases the sensitivity of the respiratory center to carbon dioxide." They overlook the possibility that sudden complete deprivation of oxygen and gradually developing anoxemia may act through different means.

We on the contrary hold that the most generally useful conception of the relations of oxygen and carbon dioxide in the control of respiration is that the supply of oxygen is the principal factor influencing the sensitivity of the neuro-respiratory mechanism. A slight deficiency of oxygen increases the sensitivity (21). Extreme deficiency on the contrary diminished the sensitivity, so that an abnormally strong stimulus (high pressure of carbon dioxide) (13) is required to excite activity. There is no other conception that covers so wide a range of facts, especially in the clinical field (22), as that—short of sudden and complete anoxia—oxygen deficiency is not a stimulus to respiration. The specific stimulus to breathing is afforded by carbon dioxide acting through the pH of the blood (23). The amount required depends on the sensitivity at the time.

After all the nerve endings in the sinus caroticus are a part of the neuro-respiratory mechanism that has heretofore been denominated as the respiratory center.

In this conception the neuro-respiratory mechanism, largely under the influence of oxygen, is considered to control the acid-base equilibrium of the body essentially as the thermal centers in the central nervous system control body temperature. A disturbance of pH is closely analogous to fever. In each form of disturbance some substance is produced in the tissues that is brought by the blood to the centers in the basal ganglia of the brain. In fever the thermal center is thereby set to maintain a higher temperature than normally. Similarly a substance (respiratory x) (9) that increases the sensitivity of the neuro-respiratory mechanism was clearly demonstrated by Geppert and Zuntz (24) to be produced in muscles during vigorous contraction. It is reasonable to assume that the same or a similar substance is produced under anoxemia. Over-ventilatoin resulting from this influence diminishes the carbon dioxide and raises the pH of the blood. A compensatory decrease of the bicarbonates in the blood follows. In intense anoxia the sensitivity of the respiratory center is depressed and the pH of the blood falls below normal. As Henderson and Haggard (9) have shown a high pH tends to "drive alkali out of the blood;" and a low pH tends to "call alkali in."

It is chiefly by such physiological reactions as these that the acid-base balance of the blood is maintained and varied under the stresses of life and in disease, rather than by mere increase or neutralization of either the acid or basic elements of the blood.

CONCLUSIONS

Diminished pressure of oxygen down to 7 or 8 per cent of an atmosphere causes a marked hyperpnea and decrease of blood alkali, but no increase of lactic acid. This fact indicates that in moderate degrees of anoxemia a formation of lactic acid is not the cause of the increase of breathing and decrease of bicarbonates.

Below 7 per cent of oxygen great increase of lactic acid in the blood develops, but in this stage respiration is not augmented. On the contrary it diminishes again to, or below, the normal volume of breathing.

Intravenous injection of sodium monoiodoacetate (33 mgm. of the acid per kilo) in normal dogs causes vigorous hyperpnea and decrease of bicarbonates, but these effects wear off within an hour.

Animals that have been deprived of the capacity to produce lactic acid by treatment with monoiodoacetate, and that are then gradually asphyxiated, exhibit essentially the same increase of respiration and decrease of blood alkali as non-drugged animals. The only considerable difference is that the drugged animals are unable to withstand as low a pressure of oxygen and to attain as low a pH as the non-drugged. This fact indicates that the asphyxial increase of lactic acid and the lowering of pH to which it contributes in undrugged animals are not toxic, but represent an effort to prevent death from failure of respiration.

In asphyxia a further lowering of pH under inhalation of 8 per cent carbon dioxide in air, instead of inducing death from intensified acidosis, promotes recovery by recalling alkali to the blood and stimulating respiration.

Acidosis in the sense of low pH is analogous to fever. The temperature of the blood is controlled by the thermal centers in the nervous system. The pH of the blood is controlled by the respiratory center. Altered sensitivity in either center expresses itself in a corresponding alteration of the blood: either its temperature or its pH.

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FECAL "FAT" AND ITS RELATION TO FAT IN THE DIET

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The source of fecal "fat" or lipid and the influence of fat in the food upon this is a subject that has received considerable attention in recent years. The old view that fatty substances in the feces represent a residue of undigested fat is no longer tenable, in the light of experimental work done principally on animals.

Bloor and his co-workers (1922, 1924) have shown that the fecal lipids of animals are excreted in constant amount and are of a uniform type, regardless of the quantity of fat in the diet or its degree of unsaturation (iodine number). Because of their similarity in composition, Sperry (1926) believes that the fecal lipids represent a secretion or excretion from the blood. This investigator showed also that the bile (1926–27), epithelial desquamation (1931) and probably bacteria (1929) are not the sole source of fecal lipids. From their work with low ileal fistulas in dogs, Sperry and Angevine (1932) conclude that the excretion of lipids originates chiefly in the small intestine and that a considerable portion is reabsorbed in the large bowel.

Comparatively little investigation has been done in human individuals to corroborate the findings obtained in animals. Friedrich Müller (1893) found that the total lipid excretion in the feces of professional fasters was approximately the same in starvation as during food periods. Voit (1892) also obtained appreciable quantities of fatty material in the excreta of starving individuals. Holmes and Kerr (1923) fed fats of high and low iodine numbers to human subjects and found that the iodine numbers of the fecal petrolic ether extracts remained the same. Their results, however, are open to criticism because they pooled the feces of their subjects before analysis. Sterile meconium obtained from new-born infants shows the presence of fatty material. Because of insufficient evidence in humans of the relation of ingested fat to that excreted from the intestinal tract, it seemed desirable to carry out experiments with this object in view.

Procedure. For this purpose, a series of 4 test diets was fed to 12 essentially normal individuals. The diets contained known amounts of fat, which were identified by their iodine absorption value. The patients

selected were surgical cases, most of whom had fractures of the lower extremities and were therefore confined to bed for 6 weeks or more. One patient was a convalescent after a mild attack of rheumatic myocarditis. All gave a history of normal digestion with daily bowel movements, before entrance to hospital. Physical examination was negative in all except two females who were obese. The age range was from 15 to 60 years. It was not possible to weigh the patients at regular intervals, but the nutrition remained good in all. The food was weighed and care was taken to see that the subjects took all of the diet. No extra food or nourishment was permitted. The patients were encouraged to drink water freely to ensure a daily bowel movement. No laxatives or oral medication were administered and, in the majority, there were one or two movements per day. Union of the fractured bones occurred in the expected length of time.

Each diet was of 6 days' duration, during the last four days of which the stools were saved. The beginning and end of each period was marked off by giving 0.3 gram carmine with the food. With the exception of case 1, all patients received the same diets, regardless of the differences in their body weight. Diet I—the so-called House Tray—consisted of fat, 107 grams (iodine no. 57); protein, 71 grams, and carbohydrate, 232 grams. Total calories 2175. Diet II was the basal low-fat diet, which on analysis yielded 8.3 grams of fat with an iodine number of 90.5. In this diet skim milk powder (0.6 per cent fat) was substituted for whole milk. Protein value was 65 grams, carbohydrate 358 grams and total calories 1766. Diet III was essentially diet II to which were added 120 grams of corn oil (Mazola) with an iodine number of 125.8. Diet IV was similar to diet III but the corn oil was replaced by cocoanut oil ("Nucoa") with an iodine number of 8.2. The carbohydrate was reduced to 232 grams in diets III and IV because of the high caloric value of the oils.

Each fresh lot of corn oil and cocoanut oil was analysed and its iodine number obtained. In the case of the former, because of its high degree of unsaturation, precautions were taken to avoid exposure of the oil to the air. The oil was therefore weighed immediately before serving it to the patients and orange juice given to mask its taste.

Case I was the first patient to whom the test diets were given. In his case diet II contained 13.3 grams of fat (by analysis) with an iodine number of 74.7. In diets III and IV 120 grams of corn oil and cocoanut oil were added respectively so that the fat intake was larger than in the subsequent cases. To one subject a fifth diet was given similar to diet III except that the corn oil was increased to 200 grams per day. Five specimens of meconium obtained from infants at birth or shortly thereafter were pooled and analysed in the same way.

Methods. In the analysis of feces Fowweather (1926) has demonstrated the superiority of the "wet" extraction method over Cammidge's

(1914) "dry" method. Lipids are more completely recovered by the former method. The technique suggested by Hill and Bloor (1922) was therefore employed.

The moist stools were weighed and transferred to a 2 litre Pyrex flask where they were kept under 95 per cent ethyl alcohol. At the completion of each test period stick KOH was added to the stools in sufficient amount to make the mixture alkaline. The contents were refluxed for 5 hours; then the flask was cooled, distilled water added and the fatty acids liberated by the addition of concentrated HCl. Extraction by repeated additions of ethyl ether was then carried out. The combined ether extracts were washed with distilled water and the excess distilled over. The residue was dried and reëxtracted with petroleum ether. The solvent was evaporated and the lipid dried and weighed. Duplicate weighed samples of the lipid were used for iodine number determinations, using the Hanus method. The diets were analysed in the same way to obtain their fat content and iodine number. Melting points were not determined: a number of the lipids were solid at room temperature—the majority, however, were liquid.

Results. In this investigation 8 of the 12 patients remained in the hospital long enough to take all of the test diets; one (case 9) took the first two diets, and three (cases 10, 11 and 12) diet I only.

"Wet" weight of feces. The "wet" weight of the freshly collected stools shows considerable variation among the group of patients, as can be expected because of the varying amounts of water in each specimen of stool. The habit of defecation differs in normal healthy adults and this applies as well to patients confined to bed. Greater or less opportunity is therefore given for the colon to reabsorb water depending upon whether the individual has one or two bowel movements per day. The exact rôle that bacteria and indigestible material play in influencing the weight of the freshly passed stool is not clear. The water content is, however, the most important factor, because it composes 70 to 80 per cent (Cammidge, 1914) or more of the undried feces. The average weights of the feces in our subjects for the 4 test diets were 507, 527, 533 and 524 grams respectively.

Weight of fecal lipids. In table 1 are recorded the weights of the lipids for each 4 day test period. Though individual variations are present, the constancy of excretion of "fat" is quite striking when one considers the differences in the amount of fat fed. The amount of fat in the food does influence to a certain extent the quantity of lipid excreted, as was pointed out by Bloor and his collaborators. They found that when dogs are given fats, the lipid excretion is larger than that obtained in starvation, sham-feeding or in fat-free diets. The same holds true, within a small range, when large and small quantities of fat are given to human individuals.

The smallest excretion occurred with the low-fat diet, larger amounts being present when corn oil or cocoanut oil were added. Further evidence is furnished by the findings in case 4, in whose diet the corn oil was increased to 200 grams per day. On analysis the feces yielded 43.1 grams lipid for the 4 day period. The excretion was larger, therefore, than that obtained on diets with less fat but the iodine number remained unchanged (64.1). If this increased lipid was due to a residue of undigested corn oil, one would expect its iodine number to approach that of the oil itself, but this was not found to be the case. It seems, therefore, that a normal individual can digest and absorb a large quantity of a vegetable fat such as corn oil with the same ease as animal fat (diet I). There appears to be no relationship

TABLE 1

Weight of fecal lipids in grams for 4 day test periods

Stool "A" corresponds to diet I, stool "B" to diet II, etc. The (+) indicates that some of the material was lost.

CASE	STOOL "A"	STOOL "B"	STOOL "C"	STOOL "D"
1*	23.5	13.0	17.0	9.8 (+)
2	18.3	15.4	19.9	14.8
3	23.9	14.9	32.9	23.4
4	12.3	10.1	13.8 (+)	16.1
5	23.8	14.7	28.6	37.1
6	15.9	10.9	24.7	14.6
7	21.7	18.0	19.7	20.3
8	22.7	13.7	37.3	12.5 (+)
9	23.9	15.9		
10	18.8			
11	15.5			
12	14.3 (+)			
Average weight	19.5	14.0	24.2	18.6

* This patient received 13.3 grams fat per day during diet II and in diets III and IV the total fat intake was 133.3 grams per day.

between the body weight of the patients and the amount of lipid recovered in the excreta. This is well exemplified in cases 6 and 7: the lipid weight in the boy weighing 45 kilos is practically equal to that of the woman weighing 104 kilos. The average weight of the lipids for the group of cases in each test period shows the relative constancy of the excretion and, when this is converted to average excretion per 24 hours, the results are even more striking. One may conclude, therefore, that in the normal state fecal lipid excretion is not greatly influenced by the ingested fat.

Iodine numbers of fecal lipids. The iodine absorption value of the lipids is shown in table 2 and the relatively slight difference in iodine number is apparent, particularly when this is compared with the degree of unsatura-

tion of the fats in the food. The variation in iodine number is much greater for the food than for the fecal lipids. Diets III and IV show the greatest contrast and were chosen because the one is a highly unsaturated product and the other practically completely saturated. The fecal lipids do not seem to have the characteristics of the dietary fat and are not greatly influenced by it, as judged by their iodine values. Even though the iodine number is slightly higher in stool C and lower in stool D, the variations from the average figure are not at all marked. The average iodine absorption value is between 40 and 50.

SUMMARY. The object of this paper has been to show that fecal lipids do not represent a residue of the fat taken in the food, when moderate

TABLE 2

Iodine numbers of fecal lipids for 4 day test periods

CASE	STOOL "A"	STOOL "B"	STOOL "C"	STOOL "D"
1*	26.0	47.5	43.4	50.7
2	45.5	55.3	52.2	59.1
3	37.1	42.6	55.9	32.9
4	47.0	63.2	67.0	50.0
5	35.6	53.5	55.1	21.7
6	39.2	56.6	69.6	59.8
7	26.2	29.2	43.2	24.9
8	41.3	59.2	42.9	38.8
9	39.1	54.8		
10	42.3			
11	40.9			
12	60.4			
Average iodine number.....	40.0	51.3	53.6	42.2

* The iodine number of the food fat in diet II was 74.7.

or even larger quantities of fat are fed. Test diets containing known amounts of fat are not quantitatively excreted but seem to be completely absorbed. This holds true equally well when 8 grams or 128 grams of fat are ingested per day. Evidence that the fecal lipids have a different composition from and remain uninfluenced by the fat in the food is also shown by their different iodine numbers. Despite the fact that the dietary fats range from almost complete saturation to a high degree of unsaturation, the excreted lipids maintain their own iodine value. As has been mentioned above, fatty substances in the stools can be recovered both in dogs and man during prolonged periods of starvation. The source of this fat is not entirely explained by epithelial desquamation, intestinal secretions and bacteria. In the newborn infant, sterile meconium contains appreciable quantities of lipid material and in 5 such specimens I recovered

a total of 1.91 grams of lipid. Eckstein (1925) has pointed out the similarity in composition of the lipids found in the feces and those present in the fat depots. It seems likely, therefore, as Bloor and his co-workers believe, that lipids from the blood plasma are excreted through the intestinal wall below its absorbing portion and are thus recovered in the feces.

CONCLUSIONS

Twelve essentially normal individuals were fed test diets containing small and large quantities of fat, and their fecal lipids were then studied. The excreted lipids were uniform in composition, as judged by their iodine numbers, and thus differed from the fats fed in which the values varied from 8.2 to 125.8. The independence of the stool lipids was further shown by the comparatively little relationship in the amounts of fat in the diet and those recovered in the feces. Fecal lipids, therefore, do not represent a residue of fat in the diet, when given in moderate amounts, but may be in part excretions of the blood into the gastro-intestinal tract.

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THE INITIAL DROP IN TEMPERATURE OF THE BLOOD IN A SUPERFICIAL VEIN OF THE FOREARM FOLLOWING A STIMULUS OF HOT WATER TO THE CONTRALATERAL EXTREMITY

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With a constant external temperature and without muscular activity, changes in the temperature of the blood in the superficial vein will depend upon the amount and velocity of blood flow. If the volume of blood is diminished, the surface cooling will be greater and the temperature of the blood in the superficial vein will therefore be lowered. On the contrary, if the blood volume is increased, the reverse will be true. Also, more rapidly circulating blood will be cooled less quickly and the temperature of blood in the superficial vein may in this manner be raised. Since a more rapid blood flow predicates a greater minute volume of blood, it may therefore be said in general that a measure of the blood temperature in an extremity is, in fact, a measure of its blood volume, at least under the conditions of the experiments here presented. Stewart (1) employed this principle in the measure of the blood volume of the hand by means of his calorimetric method. The advantage of direct measurement of the blood temperature by means of a thermo-electric couple over the calorimetric methods lies in the fact that fluctuations are more quickly discernible.

Changes in the blood flow of one extremity produced by hot or cold stimuli on the contra-lateral extremity are, as is generally known, caused by vaso-dilatation or vaso-constriction, either as the result of a skin reflex or as the result of direct changes in temperature of the blood with subsequent stimulation of the vasomotor center. Vaso-dilatation is followed by a rise in temperature while vaso-constriction is followed by a fall in temperature.

The observations which are here reported were made in the course of clinical studies dealing with vasomotor reactions of distant parts of the surface of the body to the insertion of one hand in hot and cold water.

With the subject lying quietly for an hour in a cool room (16°C.) and with a thermo-needle inserted into a superficial vein of the left forearm,

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the temperature of the blood in this vein was measured for approximately one hour before and after the right, or contra-lateral, hand was placed in hot water of 45°. A graphic record of the changes which occurred under these circumstances in the blood temperature of the left forearm is shown in figure 1-a. We note that for ten minutes after the right hand had been placed in hot water, the temperature of the blood in the vein of the left

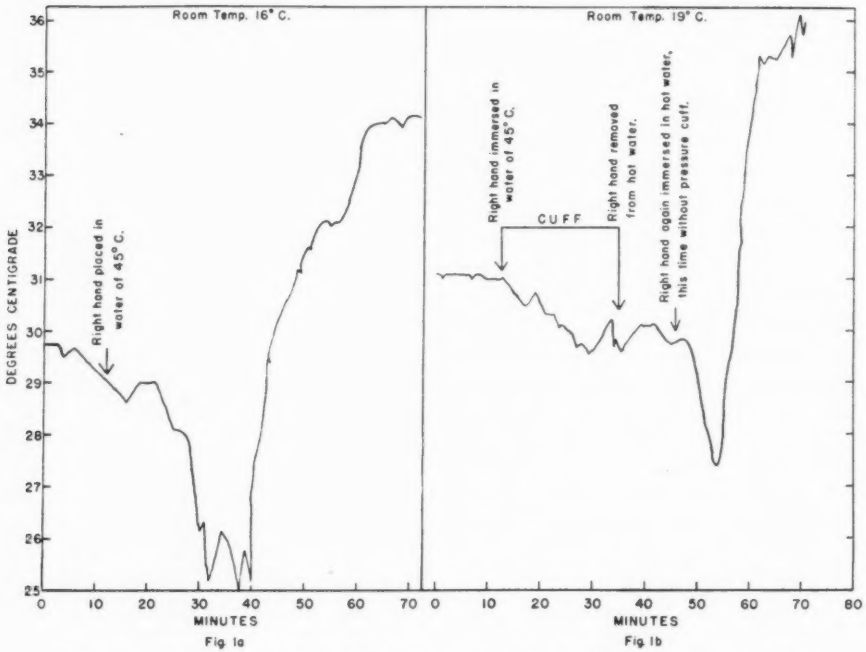

Fig. 1a. Temperature of the blood in a superficial vein of the left forearm. After the time indicated by the arrow, the right hand remained immersed in the hot water until the end of the experiment.

Fig. 1b. Temperature of the blood in a superficial vein of the left forearm. Cuff refers to the application of the cuff of a sphygmomanometer with enough pressure applied to obliterate the arterial pulsation at the wrist.

forearm remained practically unchanged. During this latent period there were slight fluctuations, but no definite tendency of the temperature to rise or fall. Following the latent period, there was first a moderate, then a more rapid and marked drop in temperature. The greatest drop had occurred in about ten minutes and amounted to 4°C. below the initial temperature. The temperature remained at this low level for approximately ten minutes and then suddenly rose to about 5°C. above the initial temperature. The

duration of this rise of some 9°C. from the lowest temperature was twenty minutes. This observation was made repeatedly on the first subject.

Further experiments were then carried out, using the same procedure in other subjects in an attempt to determine first, if the reaction is a usual one; secondly, what its mechanism is; and thirdly, its significance.

Iron-constantan thermo-elements were employed to measure the temperature of the blood. These elements were built into ordinary intravenous needles in accordance with the specifications of Sheard (2). The electrical circuit was arranged in the customary manner. A deflection of the string galvanometer of 1 cm. represented a change of 0.5°C. in temperature. The experiments were carried out in a cool room, with the temperature between 15 and 18°C. The patient lay covered with a woolen blanket, the sleeves rolled up exposing the forearms. He was asked to report any unusual subjective sensations. Before the right hand was immersed in hot water the patient lay quietly for about one hour until we were certain that the blood in the superficial veins had cooled to a fairly constant level. The low initial temperatures thus obtained permitted a wide fluctuation in temperature, from the initial vaso-constriction in the cold room to the final vaso-dilatation following immersion in hot water.

In a warm room (22 to 24°C.) the highest temperature which the blood of a superficial vein of the forearm reaches, under the conditions of our study, lies between 35.25 and 37°C. (nos. 3, 4).

Results. Eight normal persons were studied in the manner described. In each case the experiment was done at least twice. The initial blood temperatures were between 28 and 32°. In five of the eight persons there was a drop in temperature similar to that described above. The curves of the temperature fluctuations are essentially the same as in figure 1-a. In two of the persons in whom the drop was noted the experiment was repeated several times, always with the same results.

The latent period from the beginning of the heat stimulus to the beginning of the reaction varied both in different individuals and in the same individuals on different days. Usually the latent period varied between five and twelve minutes. It was found that this latency could be shortened by preliminary warming with pressure cuff applied as follows: In the experiment represented in figure 1-b the temperature of venous blood was measured in the left forearm. After twelve minutes, the opposite hand was placed in hot water of 45°, but the circulation in this arm was cut off by means of the cuff of a sphygmomanometer. At the 35th minute, 23 minutes later, the hand was removed from the hot water and the circulation was released by removing the cuff. No significant change occurred in the temperature as recorded in the other arm. At the 46th minute, the right hand was again immersed in hot water, this time without the application of the cuff. There was now the usual marked preliminary drop but after

a latent period of only 1 minute instead of the usual 7 to 10 minutes. This variation in latency may be explained on the basis of views recently presented by Pickering (5) as to the mechanism of vaso-dilatation under these circumstances. Pickering's experiments indicated that such vaso-dilatation is caused by the action on the vaso-motor center of the warmed blood returning from the extremity. This vaso-dilatation, Pickering believes, is due solely to the action of the central mechanism excited by rise of blood temperature. In the experiment here reported, the preliminary warming of the hand, with the cuff on the arm, made it possible for the blood to be warmed more quickly on the second insertion of the hand into hot water with a subsequent shortening of the latent period.

After the latent period the temperature began to drop. Usually this drop seemed to take place more slowly during the first minute or so, after which it dropped rapidly. The total drop varied between 2 and 5°C. The lowest temperature reached during such a reaction was 24°. Often the maximum low level was maintained for several minutes; in one experiment for 10 minutes.

The drop was followed by a rise in temperature, which was very rapid at first, then slower and irregular. If the heat stimulus was maintained over a sufficient length of time, the maximum temperature of 35 to 36° in the venous blood, could always be reached.

When no preliminary drop in blood temperature was observed the subject's temperature simply rose after a latent period of 5 to 10 minutes. In these cases, too, repeated experiments gave the same results.

When the room temperature was higher, 24°C., there was no effect on the blood temperature in one forearm when the opposite hand was placed in hot water. In such a case the blood temperature was between 36° and 37°C. before and after the stimulus of hot water.

It seemed likely that the preliminary drop in temperature might be due to a vaso-constriction. We were able to imitate such a drop by producing a temporary vaso-constriction by placing the contra-lateral hand in cold water of 10°. The preliminary drop in temperature to which we have been referring, if due to vaso-constriction, is a paradoxical reaction. O. Müller (6) in 1905 published plethysmographic curves showing changes in blood volume in one hand when the other hand was placed in hot or cold water. In experiments with hot water (40 to 42°) he observed a drop in the blood volume of short duration preceding the rise, which rise he assumed to be due to vaso-dilatation. He did not attempt to explain the initial drop. Martin and Jacoby (9) noted peripheral vaso-constriction following warming of a large part of the body surface. They found that if a person were placed in a bath tub filled with water of 40 to 42° in such a way that the entire lower part of the body up to the hips were covered by the water, there was, after only a few seconds, a distinct feeling of coldness

in the small of the back. The skin temperature at this spot showed a definite drop. From this drop in temperature, they concluded that a sudden hot stimulus applied to almost half of the body surface may produce vaso-constriction. It is known that any sensory stimulus of sufficient strength may produce peripheral vaso-constriction. It seems likely then that the reaction which Martin and Jacoby observed may have been due to the intense sensory stimulus of the hot water, with reflex vaso-constriction. On the other hand, the drop which O. Müller observed and for which he attempted no explanation may correspond to the drop which we

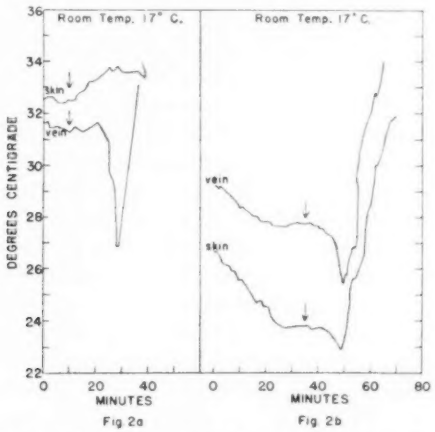

Fig. 2a Fig. 2b

Fig. 2a. Skin refers to the temperature of the skin over the left cheek. Vein refers to the temperature of the blood in a superficial vein of the left forearm. These two temperatures were taken simultaneously. The arrow indicates the time at which the right hand was immersed in hot water of 45 to 46°C.

Fig. 2b. Record of simultaneous temperature changes in superficial vein and skin of left forearm. Arrow indicates the time when right hand was immersed in water of 45°C. The right hand remained immersed in the hot water until the end of the experiment.

noted inasmuch as the stimulus was not excessive in either case. However, it is difficult to explain the very short latent period in Müller's experiments. As proof that the drop in temperature in our subjects could not have been due to the sensory stimulus of the hot water, are the following facts: The long latent period before the drop, the absence of the drop when the pressure cuff was applied over the arm which was inserted into hot water, and the absence of the drop when the initial temperature was several degrees higher.

It seemed worth while to make observations on the subjective sensations associated with the experimental procedure. There was in every case,

following the stimulus of the hot water, a generalized feeling of warmth. O. Müller (6) showed that all the surface vessels react uniformly to a hot or cold stimulus applied to any area of the skin. Pickering (5) makes the same assertion and adds that therefore the skin vessels of the hand may serve as an index of a tendency which involves the whole surface of the integument. The subjects studied usually felt the warm sensation first over the chest, then over the face and then over the entire body. These sensations occurred 3 to 5 minutes before the beginning of the drop in temperature to which we have been referring. Because of these seemingly opposing facts, we measured simultaneously the temperature of the skin over the left cheek and of the blood of the superficial vein of the left forearm, while the right hand was immersed in hot water. The results are shown in figure 2-a. It may be seen that the temperature of the skin over the cheek begins to rise within 2 minutes after the insertion of the right hand into the hot water. Five minutes later the subject had a definite sensation of warmth over the chest and face. The temperature within the vein at that time was still unchanged. Three minutes later the subject felt warm over the entire body and there were beads of perspiration over the forehead. Two minutes later while there was still a feeling of warmth over the trunk and face, the temperature of the blood in the vein began to drop. It will be noted that during this period the temperature of the skin over the cheek was continually rising. These differences, of course, are no less significant because of the fact that in one place we were measuring the temperature of the skin, whereas in the other place we were measuring the temperature of the blood; for, as can be seen in figure 2-b, the blood temperature parallels the skin temperature over a corresponding area, showing only a greater fluctuation. Some of the figures by Gibbon and Landes (8) showing changes in skin temperature under similar experimental conditions suggest the presence of such a drop of temperature in the extremities. It is likely that had the temperature of the blood been measured in their cases, a more pronounced drop would have been observed.

The results of this latter experiment, in which we measured simultaneously the temperature over the cheek and in the forearm, may be consistently explained as follows: The warm stimulus produces first a dilatation of the skin vessels of the trunk and face. The vessels of the hands are apparently more resistant to vaso-dilatation. There is then an increased vascularity of the superficial vessels of the trunk and it is conceivable that therefore there is a diminution of the blood supply to the hands. Such a blood loss might easily explain the initial drop of the temperature in the hand before the sharp drop. It is not unlikely that the entire drop may be due simply to the decrease in blood volume in the forearm as a result of the increase in blood volume in the superficial vessels of the trunk. However, another possibility must be considered: namely, that the cooling

from a blood loss may set off a reflex vaso-constriction which could then be responsible for the more precipitous drop. After a few minutes, this effect is overcome by the general spread of the vaso-dilatation and thus the temperature rises. At any rate, the results indicate clearly that the entire periphery does not always react uniformly to vasomotor stimuli and that the temperature of the skin of the hand is not necessarily an index even of the tendency of the temperature elsewhere in the skin. It appears that not only are there, as is generally known, quantitative differences in the temperature reaction of the skin of various parts of the body (9) but also under certain conditions, qualitative differences as well.

One of the 3 subjects who did not show this drop of temperature in the arm volunteered the information that while his face felt flushed, his feet were cold, though they were covered by the blanket. In this subject the experiment was repeated measuring the temperature of the blood over the dorsum of the foot instead of in the forearm. In this case, while the preliminary drop was not so pronounced, it was distinct and measured 1°C. from 26.45° to 25.4°. This drop occurred after 5 minutes. The temperature returned to the initial level in 7 minutes and then rose to 32.8° in 20 minutes.

The question arises as to why the drop in the temperature of the blood in the forearm did not take place in all subjects. Such individual variations in these as in most experiments dealing with vasomotor responses in human beings, remain unexplained.

SUMMARY AND CONCLUSIONS

1. Repeated experiments were performed on eight subjects as follows: With a thermo-couple, the temperature of the blood was measured in a superficial vein of the left forearm while the opposite hand was immersed in a warm water bath at 45°. The room temperature was between 15 and 18 degrees.

2. In 5 of the 8 subjects there was a definite drop in the blood temperature after a latent period of several minutes, followed by a marked rise in temperature.

3. Simultaneous with this drop in temperature in the forearm there was an elevation of skin temperature over the trunk and face.

4. The preliminary drop in temperature of the blood in the superficial veins of the forearm is due to a local decrease in blood volume. This local decrease, in turn, is thought to be due to generalized increased vascularity over the trunk and face. It is assumed that the cooling following the loss of blood in the forearm might produce vaso-constriction.

5. Evidence is presented which indicates that under certain conditions vaso-constriction and vaso-dilatation may occur simultaneously in different parts of the skin surface following a single vasomotor stimulus.

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LUNG EXTRACT AND BLOOD CLOTTING

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Early workers found that tissues contain a principle which promotes clotting of blood. The active principle is known as thrombokinase or thromboplastin. According to Morawitz (1), Bordet (2), and others it reacts with calcium and prothrombin, and as a result thrombin is produced. According to Howell and his co-workers (3, 4) the thromboplastin frees the prothrombin of plasma from substances which prevent its activation. The prothrombin, thus liberated, reacts with calcium to form thrombin. It is not our present purpose to discuss the relative merits of these two theories. We shall use the term "thromboplastin" without prejudice to either theory.

The work of Mills (5) brought out the fact that, as a clotting agent, lung extract is vastly more potent than is the extract of most organs. Mills and his co-workers (6, 7, 8) believed that lung extract contains a principle, "tissue fibrinogen," which unites directly with blood fibrinogen and calcium to form fibrin. This theory holds that clotting can occur without thrombin, and hence without prothrombin. This was thought to be a second type of clotting, separate from thrombin clotting. Mills assumed this second mechanism because he believed that he had excluded prothrombin from his clotting mixtures. We have reason, however, to doubt that prothrombin was actually absent. Reagents prepared from plasma can easily contain traces of prothrombin. Likewise, unperfused lung will contain some plasma, and hence some prothrombin. In preparing lung extract and fibrinogen we have taken special pains to avoid such contamination. We find that these two reagents, when carefully prepared, do not clot when mixed together with calcium, but will clot if prothrombin is added. We conclude that prothrombin is essential to clotting. We find no evidence of a second clotting mechanism.

In addition to these facts we shall present evidence to show that lung extract is extremely rich in thromboplastin. In small amounts, and in the presence of calcium, it brings about rapid conversion of the prothrombin of plasma into thrombin. In this respect it possesses a potency unequaled by extracts of all other organs which have been tested. Admittedly the lung extract contains some cephalin, but the clotting is far more rapid than that

produced by cephalin. We feel that lung deserves special recognition as a source of thromboplastin.

REAGENTS. *Lung extract.* The lung is first thoroughly perfused. It is ground, dried, and kept on ice. When needed 0.3 gram is extracted with 10 cc. saline (0.9 per cent NaCl). The pH is adjusted to 7.4 with N/10 NaOH.

$Mg(OH)_2$ suspension. Precipitate 100 cc. of 20 per cent solution of $MgCl_2$ with about 25 cc. concentrated NH_4OH. Centrifugalize and wash precipitate twice with water. Suspend precipitate in 30 cc. saline.

Adsorbed plasma. To 20 cc. oxalate plasma add 4 cc. $Mg(OH)_2$ suspension. Centrifugalize. Adjust pH of supernatant fluid to 7.4.

Fibrinogen. To 3 volumes of oxalate plasma, or "adsorbed plasma," add 1 volume saturated $(NH_4)_2SO_4$. Centrifugalize, decant, and dissolve precipitate in saline containing a little oxalate. Reprecipitate once in case of "adsorbed plasma," or twice in case of oxalate plasma. To correct for loss the final precipitate is made up to a volume one-third that of the plasma originally used. The solution is dialyzed 3 hours against saline to remove $(NH_4)_2SO_4$. The pH is adjusted to 7.4.

Fifty-six degree oxalate plasma. Heat oxalate plasma briefly to 56°C. Centrifugalize and adjust pH to 7.4 with aid of N/10 HCl.

Calcium. Dissolve 0.50 gram $CaCl_2$ + 0.525 gram NaCl in 100 cc. water. This mixture is isotonic with blood.

EXPERIMENTS. Fibrinogen is converted into fibrin through the agency of thrombin. This thrombin is formed through the interaction of calcium and prothrombin, both of which are present normally in plasma. Thromboplastin also contributes to thrombin formation. Normal circulating plasma does not contain this thromboplastin, in a free state at least. The latter is derived from injury of tissues or from break-down of blood cells and platelets. Aided by this thromboplastin the oxalate plasma clots readily if recalcified. A clotting time of 3 minutes is shown in tube 1. We wish to emphasize the fact that only a small part of the prothrombin is converted into thrombin by such traces of thromboplastin. It can be shown that over 90 per cent of the prothrombin remains, unless further quantities of thromboplastin are supplied.

Tube 1. Three drops oxalate plasma + 2 drops calcium + 9 drops saline = clot in 3 minutes.

Tube 2. Three drops lung extract + 3 drops oxalate plasma + 2 drops calcium + 6 drops saline = clot in 15 seconds.

Tube 3. Serum mixture from tube 2 + 1 drop 1.5 per cent sodium oxalate + 3 drops fibrinogen = clot in 6 seconds.

Tube 4. Three drops lung extract + 2 drops calcium + 3 drops fibrinogen + 6 drops saline = no clot in 5 hours.

Clotting of great rapidity is observed if one adds both lung extract and calcium to the oxalate plasma. Instead of clotting in 3 minutes the mixture now clots in 15 seconds, as tube 2 shows. This rapid clotting is due to the fact that lung extract causes almost all of the prothrombin to be converted into thrombin. The thrombin is rapidly formed, and, being in high concentration, it converts the fibrinogen quickly into fibrin. To show the potency of this thrombin, some of the serum mixture was taken from tube 2 immediately after the latter had clotted, and on being mixed with fibrinogen a clot formed in 6 seconds. Oxalate was added to this tube to make certain that no new thrombin was formed during this 6 second interval. We agree with Mills (9) that ordinary clotting consists of two phases. The first is an "induction" phase, during which the prothrombin is being converted into thrombin. The second is the clotting phase, during which the thrombin converts the fibrinogen into fibrin. When lung extract is added to recalcified plasma the clotting phase is seen to take only about 6 seconds. The rest of the 15 second interval is taken up by the formation of thrombin.

The above experiments show that lung extract contains an extremely potent thromboplastin which causes the prothrombin of plasma to be converted into thrombin. Tube 4 was set up to show that lung extract does not clot fibrinogen when the two reagents are simply mixed. Therefore the lung extract does not contain thrombin. Since the mixture contains calcium, any prothrombin which might be present would promptly change to thrombin. As clotting does not occur, we conclude that neither prothrombin nor thrombin is present in the mixture. Also, this experiment is incompatible with the view of Mills that lung extract contains a special factor, "tissue fibrinogen," which unites directly with calcium and fibrinogen to form fibrin. Lung extract, calcium, and fibrinogen were all present, and yet no clotting occurred. We are convinced that if clotting does occur in such a mixture it is because one or another of the reagents is contaminated with prothrombin or with thrombin.

In our earlier work we had great difficulty in obtaining fibrinogen and lung extract free of such impurities, and as a result a slow form of clotting would often take place on mixing these reagents together with calcium. We had thought all along that pure fibrinogen could be prepared by the ordinary triple precipitation of plasma with one-fourth saturated ammonium sulphate. Our first doubts on this score arose when we first made it a part of our routine to dialyze the fibrinogen against saline before using it. We observed that dialyzed fibrinogen would almost always clot in 10–45 minutes after adding pure lung extract and calcium. We soon found that the undialyzed fibrinogen contained ammonium sulphate and that for some reason the latter had been inhibiting this feeble form of clotting. After removing this salt by dialysis the traces of prothrombin present in the

fibrinogen became quite evident, and the mixture almost invariably clotted slowly on adding lung extract and calcium. It should be added that adjustment of the pH of the clotting mixtures to about 7.4 often permits clotting which would not occur otherwise.

To obtain fibrinogen of greater purity we took special care after adding the ammonium sulphate to pack the precipitate thoroughly in the centrifuge and to remove the supernatant fluid from the sides of the tube. The final product, after being dialyzed and neutralized, was sometimes of such purity that it would not clot on adding tissue extract and calcium. More often a flimsy clot formed in the course of 15–30 minutes. If this clot were removed the mixture would remain fluid indefinitely; nor would it clot on adding more tissue extract. Clearly the fibrinogen used contained traces of prothrombin. On being converted into thrombin a little fibrin separated out, carrying the thrombin with it. The rest of the fibrinogen remained indefinitely in solution, but would clot solidly if one added a little prothrombin (56° plasma).

In our efforts to purify fibrinogen we next tried repeating the ammonium sulphate precipitation 7 or 8 times, instead of the customary 3 or 4. Repeated precipitation causes part of the fibrinogen to become insoluble, but, for reasons not fully understood, this can be minimized by keeping a slight excess of sodium oxalate in the solutions throughout the entire period of preparation. The fibrinogen obtained after the 7th or 8th precipitation dissolves promptly as a rule. It coagulates quickly on adding a little thrombin. In nearly half the cases it has been freed of prothrombin to the extent that it will not clot on adding calcium and lung extract. Tube 4, already cited, is such a case.

Even with these precautions, however, the fibrinogen often still contains traces of prothrombin and will clot within 30 to 90 minutes. To obtain a better product and to do so with regularity we have finally resorted to a modification of the method recently recommended by Fuchs (10). In this method one mixes oxalate plasma, or whole blood, with a suspension of $Mg(OH)_2$. The prothrombin present is adsorbed almost quantitatively to the precipitate. The latter is then removed by the centrifuge. This clear "magnesium plasma" still contains fibrinogen, and the latter can be obtained through precipitation with ammonium sulphate. In this way one usually obtains fibrinogen of great purity. After being dialyzed and neutralized it clots promptly on adding small amounts of thrombin, but as a rule does not do so on being mixed with lung extract and calcium.

Tube 5. Three drops extract of unperfused dog lung + 2 drops calcium + 3 drops fibrinogen + 6 drops saline = clot in 15 minutes.

Tube 6. Three drops extract of unperfused calf lung + 2 drops calcium + 3 drops fibrinogen + 6 drops saline = clot in 7½ minutes.

Tube 7. Three drops extract of unperfused calf lung + 2 drops calcium + 5 drops

saline. Incubate 7 minutes. Then add 1 drop 1.5 per cent sodium oxalate + 3 drops fibrinogen = clot in 12 minutes.

Tube 8. Three drops extract of unperfused calf lung + 2 drops calcium + 5 drops saline. No incubation. Add 1 drop 1.5 per cent sodium oxalate + 3 drops fibrinogen = no clot in 5 hours.

The lung extract which we ordinarily use is made from perfused dog lung. Loucks and Scott (11) cite experiments which, we believe, indicate the need of perfusion. In our experience, post-mortem perfusion alone does not suffice. We make it a practice, in addition, to bleed the animal as a preliminary measure. At the same time we inject saline to keep up the circulation as long as possible. If perfusion of the lung is not carried out, extracts made from it will give clot formation, no matter how pure the fibrinogen may be. In tube 5 we used such an extract of unperfused dog lung. When mixed with calcium and pure fibrinogen a clot formed in 15 minutes. We have repeated the experiment, using an extract of unperfused calf lung, as advocated by Mills (6). Here again a slow form of clotting took place (see tube 6). Further study indicated that this crude lung extract does not contain thrombin. Apparently any thrombin which may have been present has disintegrated on standing. It does, however, contain prothrombin—a surprising fact in view of the enormous amounts of thromboplastin present. Apparently there is not enough calcium in the lung extract to permit conversion of these traces of prothrombin into thrombin. In tube 7 we incubated lung extract for 7 minutes with calcium chloride. As a result a definite amount of thrombin was produced, for, when reoxalated and added to fibrinogen, a clot formed in 12 minutes. Tube 8 is a control to show that the tissue extract, when oxalated, will not clot fibrinogen. We conclude that our crude lung extract contains traces of prothrombin, but no preformed thrombin.

We have attempted to purify this crude extract of calf lung, using ammonium sulphate fractionations, as Mills and Mathews (7) have done. As in their experiment, we saved the globulin fraction. After dialysis to remove the ammonium sulphate we found, however, that it still contained traces of prothrombin as well as large amounts of thromboplastin. We, therefore, consider that these efforts at purification were unsuccessful. We find it necessary to make the extracts from perfused lung, if prothrombin contamination is to be avoided.

Tube 9. Three drops lung extract + 3 drops 56° plasma + 3 drops fibrinogen + 3 drops saline + 2 drops calcium = clot in 18 seconds.

Tube 10. Three drops lung extract + 3 drops 56° plasma diluted 1–50 with saline + 3 drops fibrinogen + 3 drops saline + 2 drops calcium = clot in 7 minutes.

Finally, we have made efforts to ascertain approximately how much prothrombin is present in extracts of unperfused calf lung. As a source of

the prothrombin needed in such studies we have used oxalate plasma which had been heated momentarily to 56°C. The coagulum of fibrinogen is removed in the centrifuge, leaving most of the prothrombin in solution. We have added this 56° plasma, in varying amounts, to extract of perfused lung in order to see how much contamination is needed to give a mixture which, like crude calf lung extract, will clot in 5–10 minutes on mixing with calcium and fibrinogen. Mixing pure lung extract and 56° plasma in equal parts gives, of course, a mixture far too rich in prothrombin. As seen in tube 9 it will cause a clot in 18 seconds when mixed with calcium and fibrinogen. By trial and error we finally found that the 56° plasma, diluted 50 times with saline, contained the amount of prothrombin in question. When mixed with equal quantities of pure lung extract it is found to contain enough prothrombin to clot in 7 minutes on adding calcium and fibrinogen (tube 10). We conclude that crude lung extract contains about 2 per cent as much prothrombin as does plasma, a fact which seems reasonable enough when we reflect that lung is a very vascular organ and hence contains large amounts of blood.

Discussion. The literature contains many conflicting views regarding the nature of thromboplastin. Proteins, amino acids, alkaloids, lipoids, and fatty acids have all been studied from this viewpoint, and each has at one time or another been held to have thromboplastic power. Particular importance attaches to the work of Bordet and Delange (12), Howell (13), and McLean (14), for they have shown that phospholipin extracted from various organs contains the principle in question. The latter two workers found that the lecithin component is inert, but that cephalin is active.

We have prepared cephalin in crude form by the method of Howell and have found it to have decided thromboplastic power. If mixed in optimal quantities with oxalate plasma and calcium, a clot forms in 40 to 60 seconds. It is of great interest that lung extract acts much more quickly than does cephalin. If mixed in optimal quantities with oxalate plasma and calcium, one observes solid clotting in 15 to 20 seconds. We are unable to give a satisfactory explanation for this difference between cephalin and the thromboplastin of lung extract. We have observed that the difference between the two tends to disappear when both substances are employed at high dilution. Under these conditions the effectiveness of each is less, but we observe that the speed of clotting is more nearly equal for the two. These facts suggest that cephalin, being lipoidal in nature, and having limited solubility, is at a decided disadvantage in concentrated mixtures. In contrast, the active principle of lung extract is probably soluble, judging from the relative lack of turbidity of the extract. A soluble product might be expected to react promptly, but to be perhaps no more effective in a purely quantitative sense. It is entirely possible that the thromboplastin of lung extract is a phospholipid, such as cephalin, but

is bound to some colloid and is thus rendered soluble. On the other hand it may be that the substance is chemically quite unlike cephalin, but has merely what we might term a thromboplastic radical. However this may be, we feel that the chemical identity of thromboplastin must be kept an open question. The thromboplastin of lung extract is possessed of such potency that it deserves special consideration along with cephalin and other lipoidal substances.

SUMMARY

Thromboplastin, free of prothrombin, can be prepared by extracting perfused lung with saline. It is of great potency. Being free of prothrombin, it will not cause clotting when mixed with calcium and specially purified blood fibrinogen. This is in contrast to the view of Mills that lung contains a "tissue fibrinogen" capable of uniting directly with calcium and blood fibrinogen to form fibrin. The clotting which does occur at times is slow and is due to imperfect methods of removing prothrombin from these reagents.

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HEAT REGULATION AND WATER EXCHANGE

XVIII. The Subservience of Vapor Pressure Homeostasis to Temperature Homeostasis

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Vapor pressure mobility is essential to the most efficient temperature regulation by the mammalian organism. That such a simple fact has hitherto escaped attention must be attributed to a generally exaggerated belief in the rigidity of vapor pressure homeostasis; for the osmotic changes involved are readily detectable by the insensitive methods in common use. The vapor pressure method of A. V. Hill, however, exhibits them with considerable accuracy of detail, as will appear below.

The significance, for heat regulation, of the total blood flow through the periphery is well known; likewise the rôle herein of the relative fluid volume of the blood. These volume changes may appear in the cell counts and whole blood specific gravity, which are, of course, influenced by splenic activity as well as by actual loss of fluid from the blood stream. Volume changes are also indicated by the plasma protein concentration. This is most conveniently determined as serum specific gravity, in which acute variations indicate shifting of water with or without its content of electrolytes and more complex molecules. All the above features bear on the loss of heat by radiation, conduction and convection, but, given a reasonable minimum of peripheral blood flow, do not influence heat loss by evaporation.

Vaporization (whether by lungs or skin) depends rather on the molecular concentration of the entire *milieu interieur*. This we may properly call the vapor pressure of the body. We measure it as serum vapor pressure. To indicate its significance for heat regulation, we now report a series of experiments in which cats were exposed to various environments, cold, hot, and excessively hot.

We have previously found (1933a) in the onset ("chill period") of fever that the serum osmotic pressure increases considerably. The same change was therefore anticipated upon exposure to cold. In view of our recent demonstration of the relation of serum osmotic pressure to insensible

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weight loss (1933b) such osmotic increase would result in retarded loss of heat by vaporization.

PROCEDURE. Normal cats, fasted at least sixteen hours, after being securely fastened to boards, were allowed to rest for from fifteen to thirty minutes. From 4 to 5 cc. of blood were removed from the left ventricle immediately before and after each exposure to a new environment. The animal was immersed to the neck, in a position slightly inclined from the horizontal, in a bath at the temperature desired. All baths were continued for ten minutes. After the hot baths, the blood sample was taken without removal of the animal by simply raising it sufficiently to expose the site of puncture. After each cold bath, however, the animal with drenched skin was allowed to remain exposed to the room air for a further period

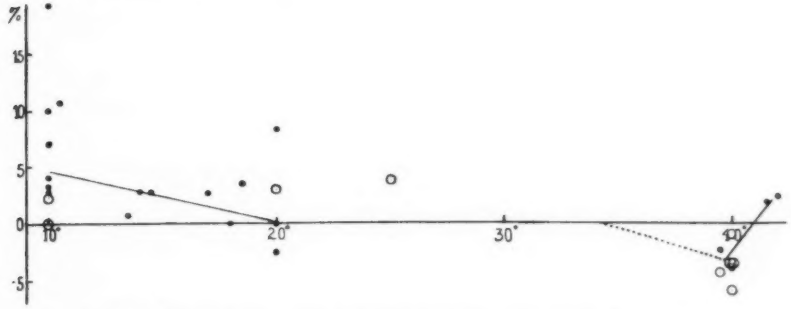

Fig. 1. The effects of cold and hot baths upon the serum specific gravity of cats. Abscissae, bath temperatures. Ordinates, per cent change in specific gravity. Each solid dot represents a primary exposure to the temperature indicated. Circles indicate the results of secondary or contrast experiments. The curve is based on primary experiments only.

of fifteen minutes. This in most cases served to increase the responses to cold.

Visible muscular activity, including shivering, was not a marked feature, and its variations can definitely be excluded as a significant influence in the results.

Serum vapor pressure was determined by the A. V. Hill method, serum specific gravity by the falling drop method, and the rectal temperature was observed at each time of blood sampling.

RESULTS. *Serum specific gravity.* To show that the fluid content of the serum, under the conditions of our experiments, responded typically to environmental changes, the results on serum specific gravity may first be presented. It will be seen in figure 1 that fluid was indeed lost from the serum under the influence of cold. Each dot represents the result of one experiment, at the temperature shown on the abscissa, the percentage

specific gravity change from the original determination being indicated by the ordinate. The threshold temperature for serum specific gravity increase under our conditions is seen to have been about 20°C., while at 10° an average specific gravity increase of 5 per cent was obtained.

Moderate increases in environmental temperature, within a narrow range, produce (as has often been shown) opposite fluid changes in the blood from those due to cold. Two new uncomplicated experiments with moderately hot baths (39.5 and 40.0°C. respectively) will suffice to show the plasma dilution. As an after-procedure in the cold bath experiments, however, five other warm baths were given. The solid dots in figure 1 represent the two uncomplicated experiments, while the circles show the results of contrasting moderate heat with previous cold.

More severe heat was introduced in two other experiments, at 41.5 and 42° respectively, which suffice to illustrate the serum concentration which results from the loss of water by heat polypnea.

The general trend of specific gravity effects we have indicated by a curve based on the solid dots only, all the circles illustrating contrast experiments. This curve is reminiscent of that which depicts the effects of environmental temperature upon metabolism; the latter tends to find its minimum in the neighborhood of the body temperature, increasing, for different reasons, both above and below this point. The same type of curve is exhibited in the third paper of this series by Lozinsky (1924), in which the whole blood solids of dogs were determined. We shall refer further on to the probable significance of the parallelism between metabolism and blood fluid changes.

Osmotic pressure. The essential findings of our present work are those which answer the questions: does the blood concentrate osmotically with cold, dilute with moderate heat, and concentrate again in a very hot environment? The following results were obtained. Five cats, exposed to 10–10.5° baths, all showed osmotic pressure increases; these averaged 7.6 per cent. The average increase for three cats at 13.5–14.5°C. was 4 per cent, and for six cats at 17–20° was 2.2 per cent. Osmotic pressure decrease in one cat at 39.5° was 4.2 per cent, while another at 40° showed a 3.5 per cent dilution. With the higher polypnea-inducing temperatures of 41.5° and 42°, increases were again noted, amounting respectively to 5.1 per cent and 4 per cent. The complete picture, including a few contrast experiments, is presented in figure 2.

Thus the osmotic concentration of the blood presents with respect to environmental temperatures a diphasic variation with the lowest point at about body temperature; the resemblance to Rubner's metabolism curve and our own curves on whole blood and plasma fluid concentrations is inescapable.

Discussion. It must be pointed out that the above described responses

to environment are truly related to heat regulation. It is true that the temperature of the animals did not remain constant in these experiments, owing to the technical convenience of keeping them in an extended position on the board. The rectal temperature always fell with the cold baths. In fact the decrease amounted to over 4°C. in some of the 10° baths. Also in the two experiments with moderate heating, the rectal temperature rose 0.5°C. each time. But the blood changes were not due respectively to cooling and heating the blood itself. They can very easily be eliminated by a variety of procedures which interfere with the central nervous system. These procedures, which will be detailed in another publication, entirely prevent both the osmotic pressure and specific gravity responses, although the blood temperature changes are even more marked

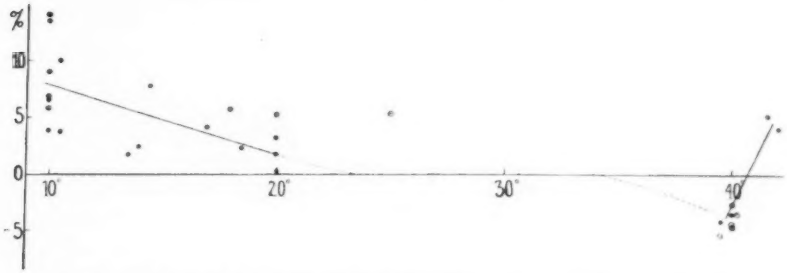

Fig. 2. The effects of cold and hot baths upon the osmotic pressure of cats. Abscissae, bath temperatures. Ordinates, per cent change in osmotic pressure. Each solid dot represents a primary exposure to the temperature indicated. Circles indicate the results of secondary or contrast experiments. The curve is based on primary experiments only.

than in our normal cats. Furthermore, the blood fluid changes can be eliminated also by deep anesthesia.

Any questions as to holding the technique of the baths, wetting the cat or excitement *per se* responsible for the results are answered completely by the fact that mild heat with the same technical procedures reverses the phenomena. As regards excitement or discomfort, both of these have also been eliminated in experiments in which the cortex has been removed, as well as in other cats with sufficiently low doses of chloralose. Neither of these procedures interferes with the responses to environmental temperature. Therefore, the response by which cold lowers the vapor pressure of the body, and by which heat raises it, is a reflex.

The basis of the osmotic pressure changes. Changes in the osmotic pressure of the serum must be accounted for in one of two general ways, either by loss of water from the extra-cellular *milieu*, or by a gain in the number of molecules or dissociated ions. In the latter case, the circum-

stances suggest first a consideration of the increase in the dextrose content of blood which is known to result from exposure to cold. In four of our experiments on 10°C. baths, the following serum dextrose increases in terms of milliequivalents were respectively observed: 4, 2.8, 4.9, and 4. The simultaneously observed milliequivalent increases in total osmotic pressure were respectively: 9, 10.5, 22, and 15. Therefore the osmotic pressure shift could only be accounted for by sugar to the extent respectively of 44, 27, 22, and 27 per cent. Blood sugar therefore in these experiments usually accounts for scarcely more than one-fourth of the osmotic pressure rise. The possibility of lactic acid increase has also been considered, although lactate is generally regarded as merely displacing carbonate upon entering the blood, and therefore not altering the osmotic pressure. In an experiment with a 14°C. bath, the initial serum lactic acid amounted to 78 mgm. per cent, which was unchanged immediately after the bath. In another experiment at the same temperature, it rose merely one milliequivalent (from 47 to 56 mgm. per cent). In a third experiment at 14.5°C. the lactic acid fell from 58 to 37 mgm. per cent. The typical osmotic pressure increase (observed also in these three experiments) is therefore not attributable to lactic acid.

Were the simple appearance in the blood stream of electrolytes or metabolites, unaccompanied by water-loss, responsible for the changes in osmotic pressure, we would still have to account for the parallel changes in specific gravity, for this feature could scarcely be affected at all by the amounts of dissolved substances concerned.

To account, therefore, for both the osmotic and specific gravity changes observed, we are thrown back upon the conception of some kind of water combination with the cells. While the question must remain undecided at present, we may look upon the analogy between the osmotic pressure curve and the Rubner metabolism curve as exceedingly suggestive. The conception of an intimate relation between osmotic pressure and metabolism is further strengthened by the fact that osmotic pressure responses as well as metabolic responses to cold can be excluded both by decerebration and by anesthesia. It is probable that increased cellular activity, involving more extensive oxidations and breakdown of metabolites, necessitates the entrance of more water into the cell at the ultimate expense of the blood plasma.

It is, therefore, reasonable to expect ultimately the demonstration that calorigenic responses to cold environment serve a dual function in heat regulation. Not only is heat production favored, but also the prevention of heat loss by evaporation.

Temperature homeostasis. While various characteristics of the mammalian body are to a remarkable degree stabilized, it does not appear to have been sufficiently emphasized that homeostasis is after all relative.

If one function tends to be thrown out of balance, the levels of others are inevitably disturbed. Thus cooling the skin upsets to a remarkable extent the metabolism, the circulation, and the water balance, and body temperature is thereby held constant.

To the list of well-known factors in heat regulation, our work has added the vapor pressure of the body and the extent of its variation in this connection is truly surprising. Why the water release from the body reservoirs should be inhibited (and even the thirst mechanism perhaps checked) by cooling the skin is as yet unknown. Why heating the skin militates against tissue hydration and especially why it seems to dull the sensitivity of the kidney to excess serum water is far from clear. However important an unchanging vapor pressure may be, it is evidently sacrificed in the service of optimal body temperature.

It is admitted that our attention has been centered upon acute phenomena in which time may have been insufficient for the usual adjustments of water homeostasis. If long continued exposure to abnormal temperatures shall be found to yield permanent readjustment of the water balance, the subservience of water to temperature control must indeed be regarded as striking. The conjecture that this finding will be made seems justified by the known adjustments of the metabolic level to climatic changes.

Temperature homeostasis in its new found relation to vapor pressure is, as in other respects, essentially dependent upon the central nervous system. Detailed evidence will soon be presented implicating the diencephalon, the sympathetic paths, and the adrenal glands. Our immediate purpose has been to establish the fact that bodily vapor pressure is subservient to environmental temperature.

CONCLUSIONS

1. Serum osmotic pressure increases in cold environments, decreases under moderate heating, increasing again with extreme heat.

2. A striking family resemblance is exhibited between the curves which relate environmental temperature respectively to metabolism, whole blood solids, serum specific gravity, and serum osmotic pressure.

3. The constancy of the vapor pressure of the body is sacrificed, not incidentally to, but in the interest of heat regulation.

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STUDIES ON COLD BLOCK IN NERVE

I. Block With and Without Freezing

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In 1930 Boyd and Gerard employed cold block in a study of excitability changes incident to fatigue in frog nerve. Low temperature was chosen in preference to other blocking agents, because of the short length of nerve required and because routine class demonstrations had shown the cold block to be rapidly reversible. This experience was confirmed in the experiments cited. Only occasionally did conduction fail to return at once on warming. In such instances recovery was found to be accelerated by washing the nerve with Ringer's solution.

The present work was originally planned as a study of this effect of Ringer's solution on the previously cooled nerve. On reviewing the literature, we were surprised to find that recovery of conduction in frog nerve, after blocking by cold, has been reported as delayed or not to occur at all (Boycott, 1902). Tait (1908) found immediate recovery in a small number of preparations, though it was more often delayed or absent. Bühler (1905) obtained recovery, usually with a greatly elevated threshold. In the light of later work, however, it seems obvious that he was not dealing with true recovery in the part frozen and then thawed, but was simply using a stimulus strong enough to spread beyond the blocked area. It is true that he found even stronger stimuli ineffective so long as the nerve was frozen. The high electrical resistance of frozen nerve, however, (Bahrmann, 1932) would probably offer an effective barrier to current spread.

The earlier workers are in general agreed that frog nerves conduct, at lowered temperatures, until they are frozen; and that freezing does not occur until the nerve has been cooled to a point varying between minus 3.0 and minus 10.0°C. The nerves blocked by Boyd and Gerard were usually frozen, at least on the surface, but measurements of temperature were not made.

I. *Block by freezing.* It will be shown below that the temperature at which a nerve freezes, and the time course of its recovery, depend mainly on the method by which it is cooled. To make this clear, a description of the apparatus is necessary.

The first device used in the present study was designed for convenience in measuring the blocking temperature. A silver tube, of about 3 mm. diameter, was fixed transversely through a paraffin moist chamber, the openings around it being sealed with vaseline. The cooling agent was a salt solution, at minus 10.0 to minus 13.0°C. The solution was siphoned from a thermos flask, through the silver tube and a rubber drain tube, the latter being provided with a screw clamp for regulating the rate of flow. To prevent the accumulation of salt above the clamp, a water trap was interposed between it and the moist chamber. By adjusting the rate of flow, the temperature on the outer surface of the cooling tube could be varied from that of the room to minus 10.0°C.

Temperature measurements were made by means of a copper-constantan thermocouple, held in contact with the silver tube by a spirally wound 18 gauge silver wire. A similar reference couple was kept in water and broken ice in a second thermos flask. Bullfrog and turtle nerves (both sciatic and median nerves from the latter) were usually wound once around the tube; the smaller pipiens nerves were simply laid across it, in the space between two turns of the wire and in contact with the thermocouple.

The thermocouples were checked at intervals. On immersion in liquids, over the range of temperature required, they were found to be accurate within plus or minus 0.2°C. Measurement of temperatures reached on local cooling of the nerve in the moist chamber was subject to additional error from conduction of heat along the wires and along the nerve. These factors were nearly constant, however, and the error involved can hardly have been greater than that of the thermometer measurements made (at greater distances from the nerve) by the earlier workers cited.

The central end of the nerve was laid over a pair of electrodes, connected to an inductorium adjusted for just maximal break shocks. The muscle twitch was the index of nerve conduction.

Freshly excised nerves of the bullfrog or turtle (tested from November 1 to May 15) always conducted until frozen. This was true also of the greater number of R. pipiens nerves (November to August, inclusive). The exceptions will be discussed below. Using preparations which had been variously treated, with the apparatus as described, we have cooled to freezing 110 pipiens nerves. The highest temperature at which any froze was minus 3.7°C., the lowest minus 9.4°, the mean minus 6.0°. This is a wide range, but it covers a comparatively large number of observations, and the figures obtained are all within the limits of those found by the earlier workers cited. Freezing was first indicated by a sudden drift of the galvanometer, showing a local rise of temperature through several degrees (cf. Bühler, 1905; Bahrmann, 1932). A few seconds later

the muscle began to twitch, but as a rule continued for a time to respond to stimulation of the nerve. The block was usually established at about the time when the muscle twitching ceased, and at a temperature still considerably above the minimum reached.

While the cooling was in progress there was always condensation of water on the metal tube. Freezing was often observed to begin among the droplets of water, with the formation of frost or of a thin layer of ice which rapidly spread to and enveloped the nerve. This made it appear possible that the block was really due to pressure from the outside. In a series of experiments, therefore, the nerve was laid on the dry metal surface and covered with vaseline before being cooled. Under these conditions, the water on neighboring surfaces sometimes froze completely, leaving the nerve unaffected and conducting well. At other times, however, the nerve froze first, leaving water still liquid a few millimeters away on the tube. Bühler (1905) discussed various possible explanations for supercooling in nerves. Since the condensed water on adjacent surfaces shows the same phenomenon, however, the explanation evidently does not involve any peculiarity of structure or of chemical composition in the nerve. Though the water froze at such low temperatures, it did not begin to melt until the thermocouple reading was approximately 0°. Our low figures are therefore not entirely due to gross error of measurement. Distilled water has been supercooled to still lower temperatures (Mellor, 1922, i, 463). In the present experiments, it seemed a matter of accident whether ice formation began first in the water or in the nerve. Afanasieff (1865), however, immersing nerves in cold oil, obtained blocking temperatures as low as minus 10.0°C.

In this first group of experiments, recovery of conduction did not as a rule take place immediately on re-warming to room temperature. Occasionally it did; but more often the block persisted until the nerve had been soaked for several minutes, sometimes for hours, in Ringer's solution. This was in agreement with Tait's (1908) observations, and entirely unlike those of Boyd and Gerard.

Attempting to find the reason for the delayed recovery, we varied the temperature of re-warming, from 20 to 35°C. The rate of cooling and of warming was also varied, but the course of recovery was not significantly affected. The experiments of the winter were repeated in June and early July, to test the possibility that recovery rate might depend upon a seasonal variation in the condition of the nerve. The summer frogs showed a somewhat higher proportion of nerves which blocked before they froze. The nerves which did freeze, however, showed the same delayed recovery that had been found earlier.

We next employed a cooling device similar to that described by Boyd and Gerard (1930). A silver rod 4 cm. long (8 gauge wire) is soldered at

one end to a tin can, the free end projecting into a moist chamber through a small hole in the wall. The nerve is laid across the rod near its free end. The can is filled with crushed ice, salt being added gradually and the mixture stirred until the nerve is blocked.

After some time we learned that with this apparatus the nerve could at will be frozen so as to show either immediate or delayed recovery on rewarming. The difference depended on the application of a vaseline seal around the cooling rod at its entrance into the moist chamber. With such a seal, the freezing mixture in the tin can had to be brought to a temperature of minus 12.0 to minus 16.0° before the nerve was frozen and blocked. Even though warming was begun at once, recovery of the nerve was usually delayed. If the opening was left unsealed, and was large enough to leave a small air space around the rod, the freezing mixture had to be cooled only to minus 8.5 or 9.0°C., and immediate recovery occurred on warming.

It thus became apparent that Boyd and Gerard had used this cooling device in such a way (without, at the time, realizing it) as to prevent or minimize supercooling of the nerve. A considerable gradient of temperature exists along the surface of the rod (depending, of course, on its diameter, temperature of the freezing mixture and of the room, protection against air currents, etc.). If it is left exposed to moist air, a film of water condenses upon it, continuous with that which covers the outer surface of the can. Ice formation begins on the latter surface, which is colder, and spreads along the rod as far as a temperature below 0° exists. Given a sufficient gradient and a continuous water film, the nerve is frozen without supercooling. A vaseline seal interrupts the film, the spread of crystallization is blocked, and supercooling takes place beyond the seal.

The first type of apparatus used had permitted supercooling to occur, because *a*, only 1 to 1.5 cm. of the cooling tube was exposed to air between the nerve and the sealed wall of the moist chamber, and *b*, the movement of cold solution through the tube tended to minimize the temperature gradient along it. We did not at first realize that these factors were of any importance. The moist chambers used varied somewhat in width, and the temperature of the cold solutions varied so that cooling to a given temperature required a more rapid flow at some times than at others. These factors may account for some of the recorded variations in freezing temperature. From the descriptions given of the cooling devices used by the earlier authors cited above, it is evident that all of them allowed supercooling to occur.

It has proved possible to modify our first type of apparatus in such a way that the nerves freeze at higher temperatures. It is only necessary to have the cold solution, before passing the nerve, flow through a sufficient unbroken length of metal tube, the outer surface of which is exposed to moist air so that a continuous film of water can condense upon it. We

have used a copper tube, of 4 mm. outside diameter and 14 cm. long, with the cold solution at minus 14 to minus 15° in the reservoir. Freezing temperatures have varied from minus 0.5 to minus 1.2°, a variation probably due largely to errors of measurement. Besides instrumental error, the movement of the ice film along the tube causes fluctuations of the galvanometer which render it difficult to make exact readings. The nerves have invariably recovered at once on warming.

Freezing of the water around the nerve is not sufficient to block it. We have seen nerves at just below 0° conducting well, though covered with ice. Nor is simple thawing sufficient to restore conduction after a nerve has been frozen. It must as a rule be warmed through several degrees, and can be blocked a second time at the same point at a temperature not low enough to freeze it (confirming Tait, 1908).

Evidently a frozen nerve is blocked, not simply by the low temperature, but by a change in its physical state. Low temperatures apparently are deleterious to the nerve only if it is frozen. We have frequently cooled nerves to minus 5.0° or lower (in two instances to minus 7.0°) and allowed them to warm again without freezing. They continued to conduct well. A nerve which has been frozen at minus 1.0°, however, shows as stated above an increased susceptibility to cold. A nerve frozen at minus 5.0° usually recovers only after a period of soaking in Ringer, and the same is true of a nerve which has been frozen at minus 1.0° and then cooled to a still lower temperature before it is thawed.

Nerves frozen at the higher temperatures present the same outward appearance as those which are supercooled before freezing. The warming drift of the galvanometer is more limited in extent. The muscle usually undergoes the same twitching before block is complete. We do not know how completely the interior of the nerve trunk is frozen.

It may be found that other factors besides freezing temperature (length of nerve exposed, time, etc.), influence the recovery. Once a nerve has been frozen, however, it is not necessary to keep it so in order to maintain the block. Such a block can be repeated several times on the same nerve, and it is rapidly reversible, provided only that supercooling is avoided.

II. *Block without freezing.* This is found, as stated above, in nerves which have recovered conduction after being frozen, and which are cooled a second time at the same point. A few freshly excised pipiens nerves, while being supercooled, blocked without freezing. There was no visible ice formation, no warming drift of the galvanometer, no muscle twitching, and there was invariable recovery when such nerves were warmed a few degrees.

This behavior was seen more frequently in nerves (from bullfrog or turtle as well as R. pipiens) dissected without the application of saline solutions, and left for some time in the moist chamber at room temperature.

The greater number of such preparations, if tested at intervals, showed a progressive rise in blocking temperature until they finally were non-irritable. The time course is extremely variable. A nerve which under these conditions has lost irritability recovers if placed for a time in Ringer's solution (we used routinely a solution containing NaCl 650, KCl 14 and $CaCl_2$ 12 mgm. per cent). If the nerve is removed from the solution at intervals and tested, the curve of blocking temperature rapidly descends until the typical freezing block occurs. The following example is fairly typical.

7/31/33. R. pipiens. Freshly excised preparation, mounted in the moist chamber at 10:52. Room temperature 30°C.
11:10. Cooled to 0° without block.
11:41. Blocked at plus 2.4°.
12:16. Blocked at plus 6.9°.
12:44. Blocked at plus 21.0°.
1:15. Non-irritable. Placed in Ringer.
1:25. Still non-irritable. Returned to Ringer.
1:35. Irritable. Cooling begun.
1:50. Blocked at plus 6.4°. Returned to Ringer.
2:04. Replaced in moist chamber. Cooling.
2:15. Blocked at minus 0.7°. Not frozen. Returned to Ringer.
2:25. Replaced in moist chamber. Cooling.
2:32. Blocked (frozen) at minus 1.1°.

In general, the rise in blocking temperature was more rapid in summer than in winter preparations, while the recovery in Ringer was more rapid in the latter. Exceptional preparations are encountered. Thus on March 10th two pipiens nerves were left overnight in a moist chamber for 16 hours. At the end of this time they were still irritable, and blocked respectively at minus 5.6 and minus 5.8°, without freezing.

The effect of Ringer's solution naturally suggests that the progressive rise in the cold-block curve may be due to drying of the nerve. No matter how rapidly the dissection may be accomplished, there is some drying, and moist chambers rarely protect perfectly against it. We believe, however, that drying is at most a minor factor. We have tested a number of nerves which had first been soaked in Ringer's solution and then exposed to room air in order to dry them rapidly. So long as these nerves remained irritable at all, they blocked by freezing. Ringer-soaked nerves were dried until non-irritable, then returned at once to the solution just long enough to restore conduction. These also blocked only when frozen.

In a series of experiments we have placed the freshly excised nerves in paraffin oil and tested them at intervals. Winter preparations behave in this medium much as they do in moist air. The following is a typical experiment.

2/6/33. Turtle, sciatic nerve.
10:10. Placed in paraffin oil.
1:38. Mounted in moist chamber. Cooling.
1:48. Blocked at plus 1.0°. Returned to paraffin oil.
2:42. Mounted again. Cooling.
2:48. Blocked at plus 3.8°. Placed in Ringer solution.
3:14. Mounted again. Cooling.
3:21. Blocked at plus 1.4°. Returned to Ringer solution.
4:28. Mounted again. Cooling.
4:45. Blocked (frozen) at minus 5.7°.

While paraffin oil should protect rather effectively against drying, it introduces another complication, namely, the possibility of asphyxia. Becht (1908) found that pipiens nerves retained irritability after immersion for five hours or more in paraffin oil. We can confirm this with respect to winter preparations, but in summer we have not found irritability retained so long.

Lack of oxygen, however, is apparently not the cause of the change in blocking temperature of nerves kept in paraffin oil. In four experiments we have used paired preparations, keeping one nerve in air and the other in nitrogen. Irritability is of course eventually lost in nitrogen, but so long as it is still present the nerve so treated shows no more susceptibility to cold than does the control nerve kept in air. The following is a typical experiment.

2/23/33. R. pipiens. Both nerves dissected without Ringer.

Nerve A left in moist chamber in air from 2:08 to 4:25, when it was blocked at plus 10.7°. Then placed in Ringer for 27 minutes, when it blocked by freezing at minus 7.0°.

Nerve B placed in moist chamber at 2:14 and nitrogen passed through until 4:34, when it was blocked at plus 11.1°. Transferred to Ringer for 20 minutes, after which it blocked by freezing at minus 5.7°.

The progressive rise of blocking temperature is not simply a manifestation of the gradual loss of function in dying tissues. Nerves kept in Ringer's solution eventually cease to conduct; but so long as conduction is possible at all we have obtained on such nerves only the freezing type of block.

It appears, then, that nerves kept in moist air undergo some change which renders them progressively more susceptible to low temperatures. The same process, carried far enough, makes the nerve non-irritable. It is not drying, not lack of oxygen, and not the gradual "death" of the nerve. It is reversible in Ringer's solution. It therefore presents certain similarities to the non-irritable state of frog muscles studied by Dulière and Horton (1929) and attributed by Horton (1930) to altered distribution of potassium within the muscle. In another paper we shall report some experiments on the influence of potassium on cold block in nerve.

It has been repeatedly observed that nerves losing irritability in the absence of oxygen may partially recover on washing with oxygen-free Ringer's solution (Thörner, 1910; Gottschalk, 1920; Cooper, 1923; Heinbecker, 1929; Feng and Gerard, 1930). But nerves kept in a moist chamber in air also lose irritability after a variable period, and these nerves also recover in Ringer. It seems possible, therefore, that in so far as conduction is restored in oxygen-free Ringer, its failure may have been due, not to exclusion of oxygen, but to the same unknown factor which caused the progressive rise in blocking temperature in our experiments.

Block of the same nerve at different levels. Garten and Sulze (1913) reported that in the nerve of a tropical frog, Rana hexadactyla, the cold blocking temperature was lower for a short stretch of nerve than for the entire trunk. This might seem to imply that conduction over cooled nerve takes place with a decrement; but such a conclusion cannot be safely drawn until it is shown that short segments of equal length, tested at various levels on the same nerve, show the same blocking temperature. If differences exist, the blocking temperature for the entire nerve could not well be lower than that of its most susceptible segment. It is a common observation that in excised nerves irritability is lost earliest near the sectioned end. We have found that, in nerves kept for some time in the moist chamber, any point near the sectioned end is likely to block at a higher temperature than points more distal. This is easy to demonstrate on a long nerve such as the bullfrog's sciatic. Examples:

5/11/33. Bullfrog nerve, dissected without Ringer and mounted in the moist chamber at 11:15.
11:24. Blocked, 2 cm. from sectioned end, at plus 0.8°. Not frozen, immediate recovery.
11:38. Blocked in middle portion, at minus 3.5°. Frozen, no recovery on warming.
11:45. Blocked 3 cm. from muscle, at minus 4.3°. Frozen, no recovery.
5/19/33. Bullfrog nerve, treated as above, mounted 2:50 and left in moist chamber.
3:42. Blocked 2 cm. from sectioned end, at 4.2°. Recovery.
3:50. Blocked in middle portion, at plus 1.0°. Recovery.
3:55. Blocked 3 cm. from muscle, at plus 0.5°. Recovery.
3:57. Placed in Ringer's solution.
4:32. Replaced in moist chamber. Cooling, 2 cm. from sectioned end.
4:43. Blocked (frozen) at minus 6.0°.

The paper of Garten and Sulze does not describe the method used for cooling the entire nerve. Presumably, the parts in contact with the stimulating electrodes and the galvanometer leads were left in air. The short intermediate segments were cooled by immersion in Ringer's solution at the desired temperature. This procedure, as shown above, would increase the local resistance to cold. The experiments therefore

do not prove that blocking temperature depends on the length of nerve subjected to cooling.

SUMMARY

1. The greater number of freshly excised frog nerves, as earlier workers have reported, conduct at low temperatures until they are frozen. Depending on the method of cooling, nerves may be supercooled to an average freezing temperature of minus 6.0°C., or supercooling may be minimized so that freezing block occurs at approximately minus 1.0°.

2. If freezing is carried out without supercooling, conduction returns at once when the nerve is warmed to room temperature. When the nerve is frozen at lower temperatures, however, recovery is usually delayed.

3. Nerves excised without the application of saline solutions, and kept in moist air or paraffin oil, show a progressive rise in cold blocking temperature, until they are non-irritable. This change takes place most rapidly near the sectioned end, so that the blocking temperature may be higher at this level than in more distal portions of the nerve. Conduction at low temperatures is restored on soaking such nerves in Ringer's solution.

4. The gradual rise of cold-blocking temperature is not due to lack of oxygen; and drying, if concerned at all, is a minor factor.

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THE EFFECT OF CARBON MONOXIDE ON TISSUE RESPIRATION

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It has been pointed out recently by Fenn and Cobb (1932a) that although it has been generally assumed that Warburg's respiratory ferment is the essential oxygen activating catalyst in all tissues, few experiments are on record in which this assumption has been put to the test in a thoroughgoing manner. A review of the literature on the subject indicated that CO does not uniformly inhibit cellular respiration.

Working with rat and with frog tissue, Fenn and Cobb (1932b) found that CO containing 21 per cent O_2, far from inhibiting tissue respiration, may actually accelerate it. This accelerating effect was obtained with all tissues except frog stomach and skin, but was exceptionally pronounced in the case of skeletal and cardiac muscle. It was also shown by direct and by indirect methods that this increased oxidation was due to the burning of CO rather than to a stimulation of tissue respiration as such.

Definite inhibition of respiration was found by Fenn and Cobb only in the case of frog skin, although when the oxygen content was reduced to 10 per cent, nerve also showed a slight inhibition. Inhibitions as high as 54 per cent were found for skin when the oxygen content was reduced to 2.3 per cent, although in these experiments with reduced O_2 tension, no data are given to show that the inhibition was not due in part to inadequate supply of oxygen. Illumination did not affect the oxygen consumption of the CO-poisoned tissues. No statement is made as to whether this factor was tried in the case of skin or nerve, where definite inhibitions were obtained.

We have performed experiments similar to those of Fenn and Cobb (1932b), using CO containing 21 to 23 per cent O_2. In essential agreement with them we find that whereas the respiration of skin, nerve, kidney and intestine is little affected, definite acceleration of oxygen consumption occurs with skeletal muscle, stomach, liver, spleen, and particularly with heart muscle.

While it is true that CO produces little or no inhibition when the oxygen content is 21 per cent, this does not exclude the possibility of inhibitions with lower partial pressures of oxygen. Hence the question originally

raised by Fenn and Cobb remains unanswered. In this paper we report experiments on the respiration of a variety of frog tissues treated with CO containing oxygen in amounts varying from 23 per cent, which is ample to supply the needs of the tissue, to 5 per cent which, with the exception of skin and nerve, is subasphyxial. The results show that CO produces clear-cut inhibitions in the case of certain tissues, such as nerve, skin, kidney, spleen, stomach, and intestine, but little or no inhibition in other tissues such as liver, heart, and skeletal muscle. When inhibition was obtained it was also possible in most cases to demonstrate partial, and in some instances complete reversal of the inhibition by illumination.

METHOD. Immediately after dissection the tissues were cut into thin slices with a razor and suspended in Ringer solution containing 10 per cent by volume of isotonic phosphate buffer (pH 7.6). An exception to this rule was made in the case of nerve and sartorius muscle; these tissues were not cut up but were used intact. The Warburg technique was used, the vessels having a capacity of about 7 cc. and containing insets for alkali and side outlets for gas perfusion. The tissue was placed in the vessel with 1 cc. of Ringer phosphate, 0.5 cc. of 5 per cent KOH being placed in the insets to absorb CO_2. Differential manometers were used throughout.

Gas mixtures to be passed through the vessels were first passed through Ringer solution contained in wash bottles submerged in the thermostat. Failure to equilibrate the gas in this manner with the vapor pressure of Ringer solution at the required temperature results in irregularities in the respiration curve.

Illumination of the tissue was accomplished by means of an arc lamp. In the first series of experiments the arc lamp with condensing lenses was placed about four feet from the thermostat to ensure sufficient spreading of the beam to give uniform illumination of the four vessels on the two manometers. The light was reflected onto the vessels by means of a mirror mounted immediately above the thermostat. It was found that this method, while fairly satisfactory, was not ideal because of the fact that the stopcock, glass capillary, and other respirometer members not immersed in the thermostat water were exposed to the intense light of the arc. Despite heat filters, this caused occasional irregularity in the respiration curve due presumably to unequal heat absoprtion.

To avoid this difficulty and to increase the available intensity of the light, the arc lamp was installed immediately adjacent to the thermostat the condensing lenses being within a few inches of the side of the thermostat. Mounted in the side wall of the thermostat was a large lens which spread the beam of light sufficiently to give a fairly uniform intensity over a wide area on the opposite side of the thermostat so that the vessels received uniform radiation throughout their area of swing. Control experiments with vessels containing Ringer solution but no tissue showed that the level

of the fluid in the manometer arms was unaffected even with the strongest illumination (100 amperes through the arc).

Five-eighths inch copper coated "Sunshine" carbons were chosen because the spectral energy distribution curve of these carbons shows a maximum at 4000 AU, which is the region where the respiratory enzyme shows a maximum absorption. If the gap between the carbons is maintained fairly uniform the average energy of the emitted light will depend within limits upon the current. It was found by control experiments both with tissue

TABLE 1

The effect of CO on tissue metabolism in the presence of a sufficiency of oxygen

EXPERIMENT NUMBER	TEMPERATURE	TISSUE	OXYGEN CONTENT OF CO	Q_{O_2}*					INHIBITION	
				Air	CO			Air	Dark	Light
					Dark	Light	Dark			
	°C.		*per cent*						*per cent*	*per cent*
23	25.0	Sartorius	23.0	0.62	0.78	0.79	0.78		−26	−27
24	25.0	Sartorius	23.0	0.65	0.74	0.72	0.74		−14	−11
25	26.3	Heart	23.0	1.60	3.38	3.48	3.18	0.90	−111	−117
26	26.3	Heart	23.0	2.11	3.66	3.93	3.63	1.22	−73	−86
37	24.7	Liver	21.2	0.64	0.77	0.82	0.69	0.58	−20	−28
38	24.7	Liver	21.2	0.86	1.12	1.25	1.12	0.81	−30	−45
39	24.0	Spleen	21.2	1.39	2.04	1.88	1.86	1.80	−47	−35
40	24.0	Spleen	21.2	1.62	1.83	2.05	1.98	1.50	−13	−27
31	22.8	Kidney	20.9	2.43	2.26	2.71	2.55	1.80	7	−12
32	22.8	Kidney	20.9	2.68	2.53	2.83	2.61	2.04	6	−6
35	22.6	Stomach	21.2	0.44	0.59	0.60	0.54	0.42	−34	−36
36	22.6	Stomach	21.2	0.41	0.59	0.61	0.55	0.35	−44	−49
33	22.4	Intestine	21.2	2.86	2.80	2.94	2.61		2	−3
34	22.4	Intestine	21.2	2.26	2.61	2.74	2.56		−15	−21
27	26.3	Skin	23.0	1.14	1.12	1.37	0.81	1.14	2	−20
28	26.3	Skin	23.0	1.27	1.16	1.43	1.22	1.17	9	−13
29	22.8	Nerve	21.0	0.36	0.33	0.37	0.34	0.27	8	−3
30	22.8	Nerve	21.0	0.32	0.29	0.35	0.35	0.31	9	−9

$$^{*}\ Q_{O_2} = \frac{O_2 \text{ (cu. mm.)}}{\text{mgm. (dry wt.)} \times \text{time (hours)}}.$$

and with yeast in CO that diffuse daylight has no photochemical effect on the CO-poisoned catalyst of respiration. A 100 watt lamp close to the vessels has some effect. Illumination with the carbon arc at 30 amperes has a maximum photochemical effect on yeast but with tissues a maximum is achieved only between 30 and 60 amperes; further increase in intensity, if anything, decreases the rate. The greater intensity of illumination required to dissociate the CO from the catalyst in tissue as compared with yeast is doubtless due to the greater thickness of the tissue slices. The

routine procedure with tissues, therefore, was to radiate with a current of 60 amperes flowing through the arc.

RESULTS. The results of the first series of experiments are summarized in table 1. In these experiments the oxygen supply was ample to meet the needs of the tissue and we found little or no inhibition by the CO. Like Fenn and Cobb (1932b) we found considerable acceleration with heart muscle and similar but smaller effects with sartorius muscle, stomach, liver, and spleen. We have never obtained the large accelerations with skeletal muscles reported by Fenn and Cobb. This may be due to the fact that the respiration rate of our preparations was very much higher than that reported by them. This disparity may perhaps have been due to a stimulating action of the phosphate buffer and to the fact that the muscles in our experiments were obtained from summer frogs. Obviously if the level of respiration is high an acceleration of oxidation due to CO burning will be masked. On the other hand, as Warburg (1927) has pointed out, unless a cell is rapidly oxidizing food material, it cannot be expected to show great inhibition with CO.

Illumination produced little effect on muscle, stomach or spleen but caused small but definite increases in oxidation in the remainder of the tissues studied.

In table 2 are summarized the experiments in which the oxygen content was decreased to subasphyxial values. In these experiments it was necessary to know the degree of asphyxia produced by oxygen lack in order to determine whether the CO was causing an inhibition. Hence the experiments were performed as follows. Immediately after dissection the tissue was divided into two equal portions (in the case of nerve, muscle, and kidney, symmetrical tissues were used as control and experimental material, respectively). The two comparable sets of tissue were then placed in vessels on separate differential manometers, the room darkened and the respiration measured in air. The values for the Q_{O_2} of the control and experimental tissues checked in most cases to within 10 per cent. Where greater differences were obtained, the disparity was probably due to slight differences in the thickness of the tissue slices or to unequal drying of the tissue preliminary to weighing. The nitrogen-oxygen mixture was then passed into the vessels containing the control tissue and the carbon monoxide-oxygen mixture into the vessels containing the experimental tissue. After obtaining the respiratory rate in the dark, the vessels were illuminated for a period of twenty to sixty minutes. After another period in the dark, air was again passed into all vessels and the respiratory rate followed in the same dark-light-dark sequence. To avoid complication, the data obtained from the latter operation are not included in table 2 (only the initial value in air is given). The experiments were performed in this manner to demonstrate that illumination has no effect on the

TABLE 2

The effect of CO on tissue metabolism under reduced O_2 tension

EXPERIMENT NUMBER	TEMPERATURE	TISSUE	CONTROL						EXPERIMENTAL						INHIBITION			
			Oxygen content of CO	Q_{O_2}					Oxygen content of CO	Q_{O_2}					Control		Experimental	
					N_2						CO							
				Air	Dark	Light	Dark	Air		Air	Dark	Light	Dark	Air	Dark	Light	Dark	Light
	°C.		per cent						per cent						per cent	per cent	per cent	per cent
59	21 7	Sartorius	9 5	0 81	0 37	0 37	0 40	0 40	9 5	0 66	0 45	0 57	0 46	0 38	55	55	32	14
13	21 5	Sartorius	5 7	0 53	0 30	0 27	0 30	0 49	6 0	0 46	0 28	0 38	0 30	0 36	43	49	39	17
11	21 0	Sartorius							6 0	0 68	0 31	0 39	0 33	0 63			54	43
10	21 1	Sartorius	5 7	0 83	0 41	0 40	0 41	0 88	2 5	0 83	0 21	0 34	0 23	0 79	51	52	75	59
60	22 0	Heart	9 5	0 62	0 55	0 58	0 55	0 56	9 5	0 72	1 35	1 57	1 38	0 62	11	6	−88	−118
12	21 0	Heart*	5 7	1 49	0 67	0 60		0 92	6 0	1 78	0 99	1 07	0 88	0 81	55	60	44	40
46	24 4	Heart	4 7	0 65	0 52	0 55	0 41	0 71	4 9	0 77	0 67	0 76	0 67	0 80	20	15	13	1
53	21 7	Liver	9 5	0 35	0 37	0 39	0 37	0 31	9 5	0 38	0 45	0 45	0 45	0 37	−9	−11	−18	−18
14	21 6	Liver	5 7	1 14	0 41	0 39	0 37	0 67	6 0	1 08	0 37	0 52	0 48	0 81	64	66	66	52
43	24 1	Liver	4 9	1 15	0 68	0 79	0 68	1 02	5 0	1 07	0 51	0 55	0 61	0 92	41	31	52	49
57	21 9	Spleen	9 5	1 89	1 65	1 66	1 75	1 90	9 5	1 51	0 97	1 51	0 88	1 39	13	13	36	0
8	21 2	Spleen							7 3	1 53	0 64	0 77	0 63	1 19			58	50
18	22 8	Spleen	6 9	0 82	0 72	0 71	0 78		6 3	0 84	0 50	0 44	0 42		12	13	40	48
22	22 7	Spleen	6 9	1 07	0 66	0 71	0 66	0 91	6 3	0 96	0 28	0 69	0 45	0 83	39	34	71	28
51	21 8	Kidney	9 5	3 78	2 89	2 88	2 70	2 76	9 5	3 62	2 11	2 74	2 01	1 87	24	24	42	24
17	22 4	Kidney	6 9	2 98	1 59	1 42	1 34	3 50	6 0	2 68	1 26	1 46	0 97	3 34	47	52	53	46
4	20 7	Kidney							5 8	2 80	0 50	1 60	1 20	2 60			82	43
47	24 2	Kidney	4 7	4 72	2 58	2 58	2 22	4 52	4 9	4 20	1 55	1 61	1 44	4 00	45	45	63	62
50	23 8	Kidney	4 7	3 72	1 84	2 14	1 74		4 9	3 70	1 22	1 73	1 41		51	43	67	53
52	21 6	Stomach	9 5	0 82	0 61	0 68	0 64	0 60	9 5	0 71	0 54	0 75	0 54	0 59	25	17	24	−5
6	21 2	Stomach							7 3	0 98	0 30	0 51	0 35	0 74			69	48
45	24 5	Stomach	4 7	0 91	0 35	0 34	0 41	0 59	4 9	0 83	0 22	0 23	0 24		62	63	74	72
56	21 8	Intestine	9 5	1 87	1 58	1 61	1 48	1 61	9 5	2 01	1 37	1 90	1 37	1 80	16	14	32	5
15	22 3	Intestine	7 9	2 08	1 38	1 29	1 29	2 34	6 0	1 56	0 60	1 00	0 60	1 72	34	37	62	36
16	22 5	Intestine	6 9	2 15	1 32	1 24	1 27	2 59	6 0	2 16	0 89	1 05	0 74	2 28	39	42	59	50
41	24 5	Intestine	4 9	1 94	0 81	0 83	0 76	1 27	5 0	2 28	0 92	1 24	0 92	2 02	58	59	60	46
54	21 6	Skin	9 5	0 72	0 56	0 56	0 61	0 60	9 5	0 73	0 70	0 70	0 68	0 61	22	22	4	4
21	22 9	Skin							6 3	0 99	0 50	0 89	0 50	1 02			50	10
5	21 0	Skin							5 8	1 30	0 62	0 83	0 60	1 22			52	36
42	24 1	Skin	4 9	0 72	0 61	0 69	0 63	0 64	5 0	0 94	0 28	0 51	0 38	1 01	15	4	70	46
58	21 7	Nerve	9 5	0 36	0 33	0 30	0 33	0 31	9 5	0 33	0 24	0 41	0 25	0 31	8	16	27	−24
20	23 1	Nerve							6 3	0 30	0 20	0 30	0 22	0 27			33	0
3	21 3	Nerve							6 0	0 29	0 14	0 20	0 16	0 16			52	31
44	24 4	Nerve	4 9	0 47	0 43	0 38	0 39	0 41	5 0	0 48	0 13	0 35	0 16	0 45	9	19	73	27

* Turtle heart.

tissue in air. In several instances it was found that light did produce a slight acceleration in oxidation of the tissue which had previously been in

CO but which was now in air. It is as though the CO union with the catalyst was not freely reversible in these cases.

Analyzing the data presented in table 2, one finds that skin and nerve stand apart from the remainder of the tissues in the degree of their inhibition with CO and in their photosensitivity. In these two tissues obviously a great part of the metabolism is mediated by an oxygen activating system similar to that which Warburg has described for yeast; this point has already been emphasized in the case of nerve (Schmitt, 1930). Intestine, spleen and kidney also show inhibitions but in lesser degree. Liver and stomach indicate some inhibition and photosensitivity but in these cases the effects are so small as to be insignificant. No inhibition could be demonstrated in the case of skeletal and cardiac muscle, indeed, even with lower percentages of oxygen, muscle shows only acceleration of oxidation under CO, as compared with the control rate for a similar percentage of oxygen in nitrogen. Heart muscle (expt. 60) shows as high as 88 per cent acceleration even in 9.5 per cent oxygen. It is interesting to note that even in the case of skeletal and cardiac muscle, illumination produces definite acceleration of oxidation.

Fenn and Cobb (1932b) found that only in skeletal and cardiac muscle does CO stimulate oxidations to any important extent. In other tissues there may be a slight acceleration but in these cases it is assumed that the rate of oxidation is the resultant of two opposing influences, an inhibition of tissue respiration by CO and acceleration of oxygen consumption due to CO burning. Since with high concentrations of oxygen we too have found evidence of stimulating effects, probably due to CO burning, it seems a safe assumption that with lower concentrations of oxygen the CO burning becomes masked by the accentuation of CO inhibition of respiration; the composite effect, therefore tends to mask the significance of both contributing factors.

Fenn and Cobb state that illumination is without effect on the rate of oxygen consumption of muscle in CO. In their experiments, however, with two exceptions, illumination consisted in exposing the vessels to diffuse daylight. It is questionable whether this is a fair test of the photosensitivity of the catalyst involved. We have tried the effect of diffuse daylight on the respiration of CO-poisoned yeast which had shown a high degree of inhibition in the dark, and found no appreciable release from inhibition, whereas illumination by the arc lamp with 30 amperes produced a prompt and maximum photochemical effect.

It is difficult to explain the data of certain of the experiments listed in table 1 and table 2 except on the assumption that illumination accelerates the catalyst of CO burning as well as the CO-poisoned oxygenase. In table 1 it is seen that while CO containing 21 per cent O_2 caused a very slight inhibition in kidney, intestine, skin and nerve in the dark, illumina-

tion not only caused the inhibition to disappear but produced a slight acceleration in oxidation. Clearer evidence is presented in the data of experiments 14, 52, 56, 41, and 58 (table 2). In these experiments illumination accelerated the rate of oxidation in the tissue treated with CO to a value decidedly higher than that of the control tissue in the nitrogen-oxygen mixture. In experiments 52 and 58 not only was the inhibition completely removed during illumination but oxidation was definitely accelerated above the original rate in air.

A possible alternative explanation of these cases may be made in terms of post-asphyxial payment of the oxygen debt. In nerve there is evidence

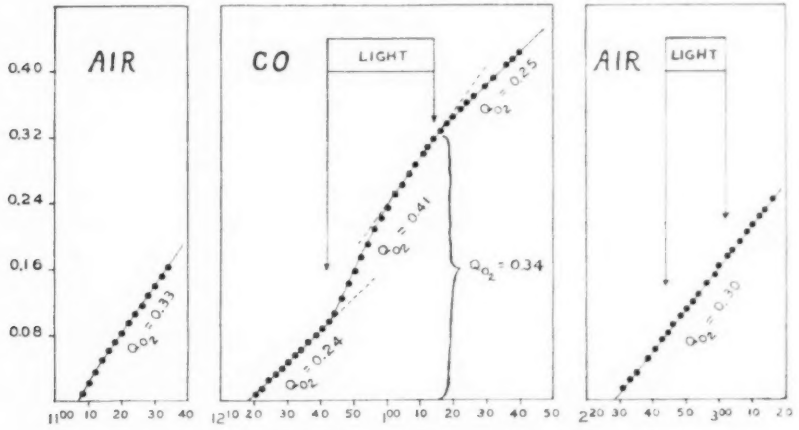

Fig. 1. The effect of CO containing 9.5 per cent O_2 on nerve respiration. Ordinates, oxygen consumption in cubic millimeters per milligram dry weight; abscissae, time. At 11:50 A.M. the CO mixture was passed through the vessels. The vessels were illuminated continuously during the interval between the vertical arrows. At 1:50 P.M. the CO mixture was replaced by air. Comparable data for the control nerves in N_2 containing 9.5 per cent O_2 are given in table 2 (expt. 58).

to indicate that the oxygen consumption after a period in pure nitrogen exceeds, for a time, that necesary for the resting demands of the tissue by an amount required to pay off the oxygen debt. This debt is interpreted as due either to an anerobic depletion of oxidative reserve or to the necessity of oxidative resynthesis of precursor substances which were broken down during anoxia (see Gerard, 1932). It is possible, then, to interpret experiments such as no. 58 as follows. During the period in CO, an oxygen debt was accumulating due to the partial asphyxia. If one assumes that during illumination the oxygenase is completely restored in activity, one would expect a preliminary period of enhanced oxygen consumption; then, after payment of the debt, the rate would return to

the normal level of the tissue. Figure 1 presents the data of experiment 58 in graphic form. It will be seen that the curve of oxygen consumption during illumination resembles closely that which one obtains by admitting oxygen to an asphyxiated nerve. In the present experiments this form of curve was obtained practically invariably with nerve. In almost half the experiments with other tissues, when definite inhibition and light recovery were obtained, the curve of respiration during illumination showed convexity downward as in figure 1.

This type of experiment furnishes valuable information concerning the oxygen debt. As regards nerve, previous experimenters have adopted the method of admitting oxygen to the vessels after a preliminary period in nitrogen. It is very difficult, however, because of vapor pressure differences of the freshly admitted gas, and because of other factors which contribute to apparatus drift, to obtain trustworthy rate readings in the first fifteen to thirty minutes after admitting the oxygen. But this is precisely the most important period because the slope of the curve of oxidation is greatest immediately after admission of the oxygen. With the CO-illumination method, as shown in figure 1 (when the dark inhibition is not too great) illumination apparently restores oxidative processes completely. Still, under these conditions, where the only change is photochemical and apparatus drift is eliminated, oxygen debts can be demonstrated to exist.

Discussion. The carbon monoxide inhibitor technique has been found a valuable tool in investigations of the oxidative nature of the processes for which certain animal tissues are especially differentiated. The fact that illumination of a tissue poisoned by carbon monoxide causes the restoration to the tissue of some measure of its normal activity—return of action potential and after-potential in nerve (Schmitt, 1930, Schmitt and Gasser, 1933), return of contractility of intestinal smooth muscle (Schmitt and Nicoll, 1933)—is evidence both of the presence in the tissue of an oxygen activating, hemin-like catalyst and of the essentially oxidative nature of a fundamental process in the function of the tissue.

From the present investigation it appears that the number of tissues which lend themselves to such a functional analysis by the inhibitor technique is somewhat limited. Nerve and skin are most suitable because of the large inhibitions and photo-restorations. Contributing to their suitability is the fact that owing to their low oxygen demand, it is possible to use high concentrations of CO without danger of oxygen lack.

With low concentrations of oxygen, and in spite of the tendency of partial asphyxia to mask the effect, intestine, spleen, kidney, and stomach show definite inhibitions with CO in the dark and partial recovery in the light. It is possible that the inhibitor technique may be of use in working out the relation between tissue function and oxidative processes in these

tissues. In skeletal and cardiac muscle the typical CO inhibition, while present, is greatly masked by the process of CO combustion.

SUMMARY

1. CO containing 21 per cent O_2 has little effect on the oxygen consumption of skin, nerve, kidney or intestine but produces definite accelerations with skeletal muscle, stomach, liver, spleen and particularly with heart muscle.

2. When the oxygen concentration is reduced to lower values, CO produces inhibition in all the tissues studied with the exception of liver, heart, and skeletal muscle.

3. Illumination causes partial, and in some cases complete recovery of respiration of the CO-poisoned tissues but has no effect on the control tissues in nitrogen containing a similar percentage of oxygen. There is some indication that the catalyst of CO combustion is also light sensitive.

4. With nerve and certain other tissues under favorable conditions the shape of the respiration curve during illumination is similar to that obtained following admission of oxygen after asphyxia, and is interpreted as representing the payment of the oxygen debt incurred during the period of partial inhibition.

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EFFECTS OF ADMINISTRATION OF PURE FOODSTUFFS AND INORGANIC SUBSTANCES ON EXTERNAL SECRETORY ACTIVITIES OF THE LIVER, PANCREAS AND STOMACH

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The present communication reports the results of studies on the composition of the biliary, pancreatic and gastric fractions of human duodenal contents obtained after the intraduodenal administration of pure foodstuffs and inorganic substances. The studies include quantitative chemical analyses of various solid constituents of the bile, estimations of the concentrations of trypsin, amylase, lipase and pepsin and of the total chlorides. The hydrogen ion concentrations of duodenal contents collected under comparable experimental conditions have been previously reported (1). For this reason such estimations were not repeated during the course of the present investigation. The purposes of the study were: 1, to ascertain the influence of administration of various substances on the character of bile, pancreatic juice and gastric contents poured into the normal duodenum of man; 2, to obtain data on the immediate origin of duodenal bile; and 3, to obtain data on the factors stimulating the secretion of bile and of pancreatic juice during normal digestion in man.

Observations have been made on the composition of human bile obtained from operative fistulae. Most of these have been confined to analyses of the lipids without reference to the effects of any particular stimulant (2). However, Pfaff and Balch (3) showed that the ingestion of whole bile was followed by an increase in the bile salt content of fistula bile. Observations which have been reported on the external secretory functions of the pancreas of man will be discussed later.

For purposes of this investigation the duodenal tube was swallowed by three apparently normal young men in the morning and 14 to 16 hours after the ingestion of food. The tip of the tube was allowed to pass into the second portion of the duodenum where its position was verified by means of the x-ray fluoroscope. Then 100 cc. of warm aqueous mixture of 50 cc. of pure cotton seed oil, or a solution of one of the following substances, 50 grams of fat-free beef peptone, 50 grams of pure dextrose, 8 grams of magnesium sulphate crystals of U.S.P. purity or N/40 HCl were slowly

run into the duodenum through the tube. During a period just prior to the introduction of a substance a specimen of the duodenal contents was collected; this represented the conditions of the fasting state. After administration of the various substances, collection of duodenal contents was begun. Collection of specimens for analysis was commenced when the light yellow bile-colored duodenal contents changed to a darker color described as some shade of brown. Such specimens were collected over periods not usually exceeding 30 minutes. The collections were continued until one of much lighter color was obtained which indicated that stimulation was diminishing or had ceased. This procedure was followed because numerous previous observations, some of which have been published (4), have established that stimulation of both the flow of bile and pancreatic enzymes is evidenced by the presence of dark colored bile in the duodenal contents. Such specimens were analyzed for the concentration of pancreatic enzymes (5), pepsin (6), chlorides (7), the furfurol number (8) which is an arbitrary measure of the bile acids, cholesterol (9) and two classes of pigments (9) differentiated by their solubilities in alcohol. Excepting the procedure for estimating chlorides, the analytical methods employed were devised by the authors. These methods were not technically involved. However, their proper performance requires considerable experience, ingenuity and judgment. When so performed they yield consistently uniform results. Obviously, the very nature of the substances estimated shows that the quantitations represent relative values only.

The findings in the duodenal bile are portrayed in a detailed outline of all the analyses of duodenal contents in chart 1. The descriptions which follow are of the findings in this chart. For purposes of description 30 minutes is designated as a collection period. For purposes of clarity the findings are schematically represented in diagrams 1, 2, 3 and 4. In these diagrams the 15 fasting contents are averaged and represent the starting point of the diagrams. The succeeding points represent the averages obtained in the three subjects of the total figures for the concentrations of a given substance in the successive specimens collected. Averages of the time of collection of specimens are also plotted. Therefore the ordinates represent concentration and the abscissae the length of time concentration was sustained.

Thirteen of the fasting duodenal contents were of light yellow color. Two specimens (expts. 3, 7, chart 1) were of dark (brownish) color due to sufficiently high concentration of an alcohol insoluble pigment (9). One specimen (expt. 7, chart 1) showed a moderate degree of concentration of solid constituents while rarely more than traces were demonstrable in the others. Excepting the dextrose experiments (expts. 7, 8, 9 of chart 1) following latent periods of 10 to 50 minutes, the concentrations of the solid elements were markedly increased after intraduodenal introduction of

CHART 1. CHARACTERS OF BILIARY, PANCREATIC AND GASTRIC FRACTIONS OF DUODENAL CONTENTS AFTER ADMINISTRATION OF COTTONSEED OIL, BEEF PEPTONE, DEXTROSE, HCl AND $MgSO_4$

SUBJECT	EXPERIMENT NUMBER	TEST MEAL	COLLECTION	COLLECTION PERIOD	VOLUME	COLOR	BILIARY FRACTION				PANCREATIC FRACTION			GASTRIC FRACTION	
							Furfurol number	Cholesterol	Pigments		Amylase	Lipase	Trypsin	Pepsin	Chlorides
									A*	B*					
			minutes		*cc.*			*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg.*	*cc.*	*mg.*	*mg. per cc.*	*mg. per cc.*
H	1	Oil	30	Fasting	42	Yellow	Tr.	Tr.	1.7	1.7	3.4	0.6	8 3	0 8	6.1
			30	1	76	Dark brown	158	103.2	18.2	18.1	3.0	1.6	7.7	0.9	3.4
			30	2	65	Dark brown	86	49.3	21.1	8.1	3.1	0.7	8.0	0.8	4 2
			30	3	57	Brown-yellow	Tr.	Tr.	7.1	2.9	3 9	0.6	5 6	0.8	7 4
R	2	Oil	30	Fasting	42	Yellow	0	Tr.	Tr.	Tr.	3 2	1.0	2 1	0 9	6 0
			30	1	38	Brown	26	25.9	8.4	2.7	4.2	1.2	5.7	0.6	7 0
			30	2	48	Brown	34	40.1	13.0	6.1	4.5	1.5	8.6	1 0	5 3
			30	3	52	Brown	44	39.0	12.8	4.0	4.0	1.2	6.9	1.0	5 2
A	3	Oil	30	Fasting	80	Brown-yellow	Tr.	Tr.	4.1	Tr.	1.9	0.5	2 7	2 2	4 9
			30	1	46	Brown	180	150.0	23.1	17.2	4.8	2.3	9.2	2.6	4.2
			30	2	53	Brown	110	50.0	17.7	12.3	4.7	1.0	5.4	2 9	5 0
			30	3	100	Brown-yellow	Tr.	Tr.	4.3	Tr.	3.0	0.4	2 8	3.1	5 0
H	4	Beef peptone	30	Fasting	73	Yellow	Tr.	Tr.	Tr.	0.3	0.3	0 6	12.4	12 0	6 8
			15	1	58	Brown-yellow	52	15.5	3.3	4.2	2.2	1.4	5.2	5 2	7 0
			13	2	64	Yellow	Tr.	Tr.	Tr.	1.3	1.1	1.2	3.2	15.7	7.6
			30	3	74	Yellow	Tr.	Tr.	Tr.	0.9	1.6	1.5	5.1	13 4	7 4
R	5	Beef peptone	30	Fasting	17	V. L. Yellow	0	Tr.	Tr.	Tr.	1.9	1 2	1 3	0 7	6 1
			30	1	43	Brown	68	44.2	30.0	6.9	3.6	0.9	9.5	2 0	8 5
			30	2	31	Yellow	0	Tr.	Tr.	Tr.	4.3	0.3	2 6	1.7	5 4
A	6	Beef peptone	30	Fasting	32	Yellow	18	Tr.	Tr.	Tr.	0 9	0.1	2.4	0 5	4 7
			16	1	62	Brown	100	60.0	10.0	10.2	1.0	1.1	3.2	2 2	5 3
			14	2	104	Yellow	22	54.2	2.3	2.3	0.0	0.1	0 5	9 6	6 8
			30	3	28	Light yellow	Tr.	Sl. tr.	Tr.	Tr.	0 0	0.1	0 5	6 0	7 7
H	7	Dextrose	30	Fasting	66	Dark yellow	20	20.9	4.1	1.5	2.1	0.8	10.6	1 4	5 6
			30	1	130	Yellow	Tr.	Tr.	2.0	0 7	0.9	0 2	2 8	0 5	3 4
			30	2	31	Brown	Tr.	12.5	6.4	3.0	2.4	0.3	5.1	0 9	4 8
			30	3	47	Yellow	Tr.	Tr.	3 3	1.5	2.9	0 2	4 8	1 0	4 2
R	8	Dextrose	30	Fasting	13	Light yellow	0	Tr.	Tr.	Tr.	2 4	0 2	3 0	0 7	6 5
			30	1	51	Brown	Tr.	26.8	7.5	2.1	0.7	0 5	3 3	1 6	4 1
			45	2	18	Yellow	Tr.	33.1	Tr.	Tr.	2.6	0 8	4.7	1 5	7.2
A	9	Dextrose	30	Fasting	31	Yellow	Tr	0	Tr.	0 1	0.8	0.4	2.6	0.5	5 7
			30	1	85	Dark yellow	Tr.	0	0 9	0 2	0.2	0 2	1 3	0.6	2.7
			30	2	77	Light yellow	0	0	0	0	0 5	0 3	1 1	2 1	3 5
H	10	HCl	30	Fasting	72	Yellow	Tr.	11 1	1 7	Tr.	3 8	0 5	4 5	0 9	6 2
			8	1	81	Brown	48	26.1	5.5	4.2	1.0	1.2	2.8	0 8	3 2
			30	2	104	Yellow	24	10.7	3.5	1.7	2.0	1.1	3.5	0 7	6 4

CHART 1—*Concluded*

SUBJECT	EXPERIMENT NUMBER	TEST MEAL	COLLECTION	COLLECTION PERIOD	VOLUME	COLOR	BILIARY FRACTION				PANCREATIC FRACTION			GASTRIC FRACTION	
							Furfurol number	Cholesterol	Pigments		Amylase	Lipase	Trypsin	Pepsin	Chlorides
									A*	B*					
			minutes		*cc.*			*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*mg.*	*cc.*	*mg.*	*mg. per cc.*	*mg. per cc.*
R	11	HCl	30	Fasting	18	V. L. Yellow	0	0	0	0	2.9	0.8	4.2	1.0	5.9
			30	1	151	Brown-yellow	38	52.8	4.9	2.7	0.7	0.8	3.8	0.7	4.2
			30	2	32	Yellow	0	Tr.	Tr.	Tr.	2.1	0.8	3.7	0.9	6.8
A	12	HCl	30	Fasting	28	Yellow	Tr.	Tr.	2.0	0.4	1.3	0.2	2.6	0.3	6.4
			30	1	135	Brown	52	30.3	15.8	3.3	0.9	0.8	3.2	0.2	3.6
			30	2	41	Brown-yellow	30	Tr.	5.7	1.9	1.9	0.5	4.1	0.3	8.0
H	13	$MgSO_4$	30	Fasting	85	Yellow	Tr.	Tr.	Tr.	Tr.	0.1	0.6	2.2	12.9	6.6
			14	1	35	Brown	40	29.7	7.6	2.5	1.2	1.1	3.7	1.1	6.4
			16	2	64	Yellow	22	14.3	1.9	1.1	1.5	1.3	4.1	1.0	7.0
			30	3	32	Light yellow	Tr.	Tr.	Tr.	0.2	1.8	1.2	3.6	1.0	7.2
R	14	$MgSO_4$	30	Fasting	35	Yellow	Tr.	Tr.	Tr.	0.8	3.6	0.2	3.3	0.8	6.4
			20	1	47	Brown	64	22.5	3.0	5.7	0.9	1.0	4.6	0.9	5.2
			30	2	48	Yellow	36	10.2	2.0	1.4	1.4	0.7	3.9	1.1	7.0
A	15	$MgSO_4$	30	Fasting	22	Yellow	Tr.	Tr.	Tr.	Tr.	1.0	0.2	2.2	0.5	4.8
			25	1	83	Dark brown	94	51.0	11.1	11.2	1.4	1.0	4.4	0.7	4.6
			30	2	75	Dark yellow	32	16.7	5.6	3.2	1.5	0.9	4.4	0.8	4.4

A* Alcohol insoluble pigment. B* alcohol soluble pigment.

foodstuffs and the inorganic substances, as the diagrams show strikingly. Oil (expts. 1, 2, 3 of chart 1) produced fairly well sustained concentrations throughout two or three experimental periods. Peptone was followed by concentrated bile one-half, one and two experimental periods respectively in experiments 4, 5 and 6 of chart 1. Dextrose produced very moderate stimulation of concentrated bile in only one experimental period (expt. 8, chart 1). HCl produced concentrated bile for one period in one experiment (expt. 11, chart 1), and for 2 periods in experiments 10 and 12, chart 1. $MgSO_4$ produced two periods of concentrated bile in all experiments (expts. 13, 14, 15, chart 1). Thus, concentration of bile was sustained longer after oil than after any other substance, as is well illustrated in the diagrams. $MgSO_4$ and next HCl solutions sustained bile concentration better than did peptone, while dextrose was neutral in its action. Excepting the one period of stimulation following the administration of peptone (expt. 5, chart 1), oil gave the greatest concentration of bile of any foodstuffs fed to the same subject; peptone administration gave the next greatest stimulation, and dextrose the least. The degrees of concentration of bile constituents following administration of HCl and $MgSO_4$ were fairly

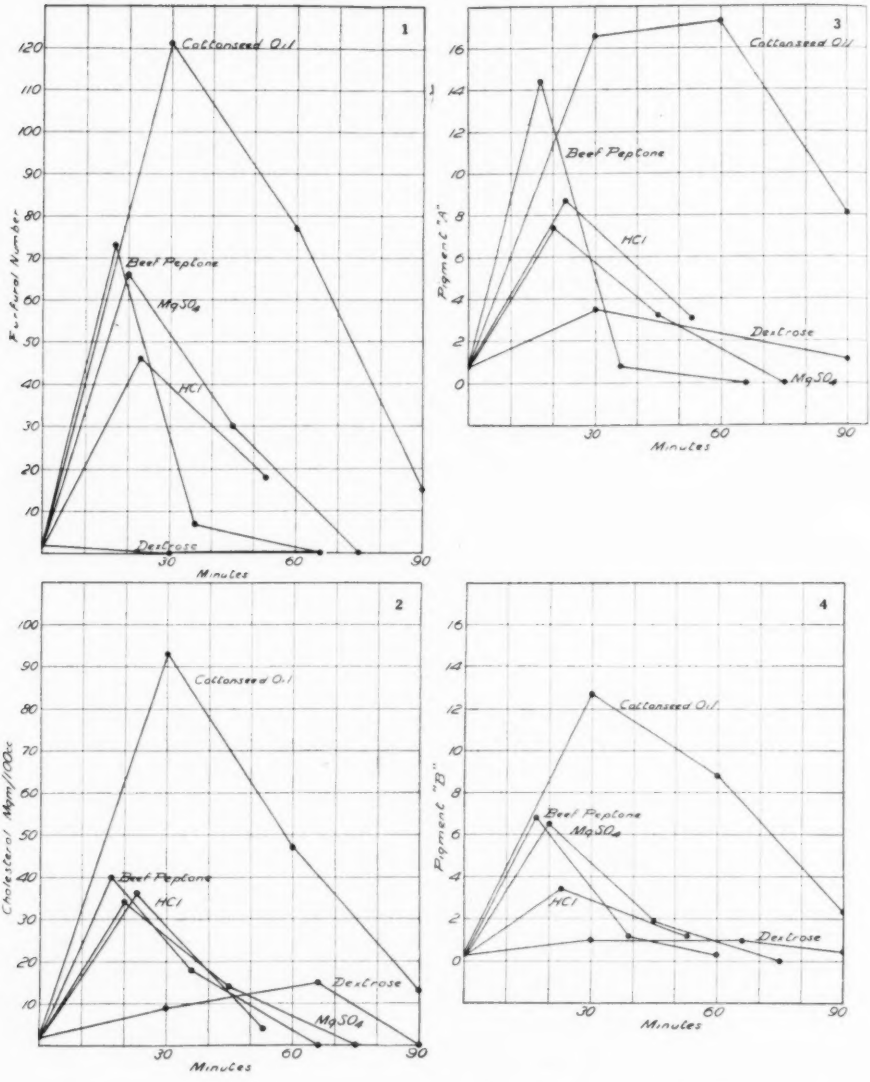

Diagram 1. Average concentrations and durations of stimulation expressed as furfurol number.

Diagram 2. Average concentrations and durations of stimulation of cholesterol concentrations.

Diagram 3. Average concentrations and durations of stimulation expressed as concentrations of pigment A.

Diagram 4. Average concentrations and durations of stimulation expressed as concentrations of pigment B.

comparable and a little less than those following peptone. The findings show that oil is the most powerful factor in producing concentrated bile in the duodenum, while products of protein decomposition, HCl and $MgSO_4$ produce bile of comparable concentration. Dextrose produces very moderate or no increase in bile concentration while dilute bile was persistently found during the fasting state. All these interpretations of the findings are well illustrated in diagrams 1, 2, 3 and 4.

During stimulation (chart 1) the volumes varied from 35 to 151 cc. (expts. 13 and 11, chart 1). Following stimulation the amounts varied from 35 (expt. 13, chart 1) to 100 cc. (expt. 3, chart 1). Relatively large volumes, up to 130 cc., were found after administration of dextrose without stimulating effects (expt. 7, chart 1). The fasting amounts (chart 1) also varied considerably. The volumes of 4 fasting contents were 13, 17, 18 and 22 cc., those of the others varied from 31 to 85 cc. In five experiments (expts. 2, 3, 4, 10, 13, chart 1) the fasting contents were as great in volume as after stimulation with one of the substances, and in a sixth (expt. 14, chart 1) the fasting contents approximated the volume obtained following stimulation. Further discussion will be undertaken later.

Reliable chemical methods for estimating the amounts of fatty acids (10) and phosphorus (11) and the bromine number (10) of the fatty acids of bile have been developed by the writers. Studies of the effects of the intraduodenal administration of cottonseed oil, of the peptone and dextrose solutions on the concentrations of the total fatty acids and of phosphorus of duodenal bile have been made by the use of these methods. The findings will be reported fully elsewhere. Those of value in relation to the present investigation will be briefly discussed later.

Because of their bearing on the origin of duodenal bile, observations were also made on selected subjects whose gall bladders had been excised. The results obtained by the intraduodenal administration of cottonseed oil or oleic acid on the concentration of the solid constituents of duodenal bile are outlined in the following table.

The first patient (table 1, Hud.) had had the gall bladder removed and at the operation an early cirrhosis of the liver was discovered. Nevertheless, the figures for furfurol number and for cholesterol are more than those for the corresponding period in the normal subject (expt. 2, chart 1) after oil administration. When compared with established arbitrary normal levels, case 23 shows a high concentration of cholesterol and of alcohol soluble pigment "B" and a moderately diminished furfurol number; while case 28 is distinguished chiefly by a concentration of alcohol insoluble pigment "A" well above the established minimum normal limit (4). In patient Hud. the total fatty acids found in duodenal bile after stimulation with oil was 6.7 mgm. per 100 cc. of bile and in one normal (expt. 2, chart 1)

it was 9.4 mgm. The bromine numbers of the total fatty acids of these specimens showed that they contained none of the oil administered. In addition to the patients studied in table 1 the duodenal bile was obtained from an adult prior to cholecystectomy and from a common duct fistula after the operation. On laparotomy no evidences of gall bladder or other intra-abdominal disease was found. The duodenal bile was obtained after stimulation with oleic acid. Bile was collected from the common duct

TABLE 1

Effects of intraduodenal administration of cottonseed oil or oleic acid on composition of duodenal contents after cholecystectomy

SUBJECT	TEST MEAL	COLLECTION	COLLECTION PERIOD	VOLUME	COLOR	FURFUROL NUMBER	CHOLESTEROL	PIGMENTS		PANCREATIC ENZYME CONCENTRATIONS			PEPTIC CONCENTRATION	CHLORIDE CONCENTRATION
								A*	B*	Amylase	Lipase	Trypsin		
		minutes		*cc.*			*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*					
Hud.	Cottonseed oil	30	F	76	Yellow	Tr.	Tr.	Tr.	Tr.	0.4	0.4	0.7	1.2	6.0
		20	1	58	Yellow green	24	17.3	Tr.	1.8	1.0	1.0	2.2	0.7	5.4
		30	2	30	Yellow green	30	28.5	0.0	1.8	—	—	—	—	—
		30	3	56	Yellow green	38	21.2	1.5	1.1	2.2	1.2	3.9	1.0	6.8
		19	4	24	Yellow green	24	11.0	—	—	2.7	2.0	4.0	7.9	6.8
23	5 cc. oleic acid	30	F	40	Yellow	Tr.	Tr.	Tr.	Tr.	—	—	—	—	—
		30	1	55	Brown	56	84.0	4.0	12.3	2.4	0.3	3.5	—	—
28	5 cc. oleic acid	30	F	35	Yellow	Tr.	Tr.	Tr.	Tr.	—	—	—	—	—
		30	1	50	Brown	32	18.0	5.7	0.3	1.5	0.0	1.3	—	—
Pat.	Cotton seed oil	30	F	100	Yellow	10	Tr.	Tr.	1.0	1.1	0.3	4.7	0.9	4.4
		17	1	20	Yellow	26		Tr.	1.4	2.3	0.6	6.7	0.8	5.2
		28	2	53	Yellow	16	Tr.	Tr.	0.9	1.3	1.0	5.1	0.7	3.8

A* Alcohol insoluble pigment.
B* Alcohol soluble pigment.

fistula after the patient had sufficiently recovered from the operation for cholecystectomy to eat the usual general hospital diet. The specimens analysed represented a collection of bile after ingestion of 50 cc. of cottonseed oil followed by 100 cc. of water. Analyses of these biles are shown in table 1.

The duodenal bile is a fairly concentrated one as compared with the arbitrary normal (4) for the experimental conditions employed. When compared with the findings in the duodenal biles outlined in chart 1 or the

preceding duodenal bile from the same subject the fistula bile was fairly highly concentrated. Several such concentrated fistula biles were collected from this same subject. The findings discussed in tables 1 and 2 show that in the absence of the gall bladder the duodenal bile of selected subjects may contain various constituents of the bile in relatively high degrees of concentration. The biliary function of the liver of such patients as these is abnormal and is usually depressed (12) as represented by case Pat. of the table. The abnormal liver functional state explains the low concentrations found for certain of the solid constituents in the specimens of the three subjects (Hud. 23, 28 of table 1).

The findings described above present various data the interpretation of which is of much importance. Among these is the question of the origin of duodenal bile obtained during the fasting state as outlined in chart 1 and diagrams 1, 2, 3 and 4. During the entire periods of collection of such

TABLE 2

Duodenal bile obtained prior to and fistula bile after cholecystectomy on a non-demonstrably diseased man

SPECIMEN	FURFUROL NUMBER	CHOLESTEROL	PIGMENTS	
			A*	B*
		mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*
Duodenal	108	67 8	5 9	4 5
Fistula	104	15 7	29 2	13 3

A* Alcohol insoluble pigment.
B* Alcohol soluble pigment.

bile the contents returned through the duodenal tube were persistently colored with bile pigment. That the latter was the result of an immediate ejection of bile from the common duct is evidenced by the fact that return of duodenal contents through the tube depended on peristalsis of the duodenum, which action kept that region free from stagnation. If the bile had come from the gall bladder, obviously that organ must have been emptying at intervals of a few seconds to a minute which as far as is known it does not do in the fasting state (13). There was, furthermore, no evident factor present which would stimulate the gall bladder to empty its contents. These findings indicate that the fasting bile represented a secretion coming directly from the liver.

The origin of the concentrated bile which followed the administration of the various substances is of even more importance than that obtained during fasting. Ivy (14) discusses analyses of duodenal bile in a résumé of the evidence of evacuation of the gall bladder into the common duct and gives no consideration to the possible secretion of concentrated bile by the liver.

Physiologists in general hold the opinion that concentrated bile comes only from the gall bladder (14), although no one presents experimental data to adequately support the opinion that duodenal bile is composed either exclusively or largely of gall bladder bile. Indeed, until very recently the whole conception that duodenal bile originated in the gall bladder was based solely on the fact that that vesicle contains concentrated bile. The interpretation of findings of the experimental work presented here will be based on the premise that the gall bladder evacuates its contents into the common bile duct. The first question discussed is whether duodenal bile represents an accumulation in the gall bladder occurring prior to the time of the experimental period as outlined in chart 1. Data obtained during the course of the present investigation furnish information on this subject. Such data comprise the bromine numbers of the total fatty acids and the proportional relationships of the concentrations of solid constituents of specimens of bile collected successively during the period of flow of relatively highly concentrated bile. These data have been correlated in table 3, which follows. The table represents all those experiments in which two successive collections of duodenal bile were made during the period of stimulation. Studies on the lipids of duodenal bile were not made after administration of inorganic substances.

Study of the table shows the bromine numbers of the fatty acids in experiments 1, 4, 5, and 9 varied sufficiently in successive specimens to demonstrate the presence of fatty acids of different chemical composition in these specimens. Obviously, if the bile had come exclusively from the gall bladder the fatty acids of all successively collected specimens would necessarily be of the same chemical composition. The table outlines also both the absolute and percentage changes in the concentrations of the solid elements. The specimens of duodenal bile in which the bile acids, cholesterol and pigment concentrations were determined were collected on different days than those in which estimations were made of the total fatty acids, organic phosphorus and bromine numbers. Study of the relative increases or decreases of the furfurol number, cholesterol and pigments in the same successive specimen shows that these did not occur proportionally. Striking examples of this occur in the table (table 3). In the HCl experiment 7 the furfurol number and soluble pigment decreased 42 per cent, alcohol insoluble pigment 64 per cent and cholesterol 100 per cent. In the peptone experiment 6 cholesterol decreased but 9 per cent while all other elements diminished 77 per cent. In the oil experiment 9 three elements decreased 40 to 55 per cent while the alcohol insoluble pigment increased 14 per cent. The other set of duodenal biles shows great differences between the proportional decreases of total fatty acids and organic phosphorus in the same successive specimens. Obviously, if the bile specimens analyzed had originated exclusively from the gall bladder changes in the

TABLE 3

Proportional relationships of solid constituents of successive specimens of duodenal bile

SUBJECT	EXPERIMENT NUMBER	TEST MEAL	FURFUROL NUMBER	CHOLESTEROL	PIGMENTS		TOTAL FATTY ACIDS	ORGANIC PHOSPHORUS	BROMINE NUMBER FATTY ACIDS
					A*	B*			
				mgm. per 100 cc.	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	*mgm. per 100 cc.*	
R	1	Cotton seed oil	26	25.9	8.4	2.7	9.36	0.179	29.1
			34	40.1	13.0	6.1	3.05	0.103	26.1
			+30%	+55%	+60%	+80%	−67%	−42%	
			44	39.0	12.8	4.0			
			+69%	+50%	+52%	+50%			
R	2	Casein peptone					1.75	0.214	37.0
							1.03	0.153	35.7
							−41%	−28%	
R	3	$MgSO_4$	64	22.5	3.0	5.7			
			36	10.2	2.0	1.4			
			−44%	−54%	−33%	−74%			
A	4	Cotton seed oil	180	150.0	23.1	17.2	13.55	0.436	34.5
			110	50.0	17.7	12.3	2.33	0.264	20.8
			−37%	−66%	−23%	−28%	−83%	−40%	
A	5	Dextrose					2.47	0.066	26.7
							2.57	0.071	32.1
							−36%	+0.7%	
A	6	Beef peptone	100	60.0	10.0	10.2			
			22	54.2	2.3	2.3			
			−78%	−9%	−77%	−77%			
A	7	HCl	52	30.3	15.8	3.3			
			30	Tr.	5.7	1.9			
			−42%	−100%	−64%	−42%			
A	8	$MgSO_4$	94	51.0	11.1	11.2			
			32	16.7	5.6	3.2			
			−66%	−67%	−50%	−70%			
H	9	Cottonseed oil	158	103.2	18.2	18.1	3.29	0.095	32.4
			86	49.3	21.1	8.1	20.30	0.062	24.6
			−40%	−52%	+14%	−55%	+52%	−33%	
H	10	HCl	48	26.1	5.5	4.2			
			24	10.7	3.5	1.7			
			−50%	−59%	−36%	−60%			
H	11	$MgSO_4$	40	29.7	7.6	2.5			
			22	14.3	1.9	1.1			
			−45%	−52%	−75%	−56%			

A* Alcohol insoluble pigment.

B* Alcohol soluble pigment.

concentrations of solid elements of the same successive specimens would necessarily be approximately the same. The variations found in the solid constituents quantitated present further evidence that duodenal bile is not derived solely from the gall bladder. Indeed these discrepancies were so great that there is very little evidence that bile from the gall bladder was an important factor in influencing the concentration of duodenal bile.

The findings discussed show that the composition of successively collected specimens of duodenal bile is such that it is highly improbable that its concentration was greatly influenced by action of the gall bladder. It was also shown that concentrated bile (tables 1 and 2) can be secreted in the absence of the gall bladder. Furthermore, Mendenhall has shown that $MgSO_4$ (15), oil (16) and peptone (16) have a marked cholagogic effect in animals. These findings indicate, therefore, that under the stimulating effects of oil, peptone, HCl and $MgSO_4$ the concentration of the solid constituents of bile found in the duodenum depended more on the character of bile coming directly from the liver than on bile evacuated from the gall bladder.

The variations in biliary constituents discussed, and outlined in table 3, may be interpreted as have the findings in lower animals. Whipple (17) studied the excretion of pigment in the fistula bile of dogs. Although the bile was subjected to the concentrating action of the gall bladder (18) it was concluded that bile pigment excretion is influenced by various foodstuffs. Observations have been made on the influence of various substances on the composition of bile which has not been influenced by the action of the gall bladder. Rous, Broun and McMaster (19) confirm the finding of Hooper and Whipple (17) that dextrose administration to dogs produces temporary increase in the output of bilirubin. This output is possibly "consequent on the deposition of glycogen in the liver." It is not due to "any consistent alteration in the rate of bile flow, which furthermore is almost regularly less on a carbohydrate diet than on one of meat" (19). Feeding bilirubin to dogs greatly increased the concentration of this pigment in hepatic bile. On the other hand, feeding bile salts caused no change in the pigment but it greatly increased the quantity of bile (20). It has also been shown that day to day variations in the output of bile pigment result from changes in the blood (21). Feeding cholesterol to dogs increases the quantity of that substance eliminated in the bile. Cholesterol excretion is an independent functional activity of the liver not related to the quantity of bile or of bilirubin (22). Whipple and his associates found that the output of bile salts depended on the secretory activity of the liver and that their concentration in the bile was influenced by different types of foodstuffs (23), (24). They also showed that the amount of bile is increased by feeding bile or bile salts (25), certain meat proteins (24) and some amino acids (26). Calcium is an excretory product of the

liver and its amount of bile does not depend on the volume of the latter (27). Other studies have shown that the composition of hepatic bile is influenced by operative interference, hot weather, and various normal and pathological conditions (28). These observations show that the liver possesses several biliary functions which act largely independently of each other. They also show that the liver has the ability to so modify the activities of these functions as to change the volume of bile and the degrees of concentrations of its solid constituents.

The experimental results present data bearing on the question whether the bile whose flow has been stimulated under the experimental conditions described passes through the gall bladder on its way to the duodenum. If such passage were the only factor which produced the concentration of duodenal bile it would be anticipated that all specimens collected would be of approximately equal concentrations, which was not the case. If only a part of the bile passed through the gall bladder then in order to explain the effects of different stimuli on bile concentration obviously there must exist some mechanism which controls differentially the amounts of bile diverted into the gall bladder under the influence of these different types of stimulants. Such a mechanism could be hypothecated as the result of selective control of the sphincter of Oddi by the different types of stimulants employed for the proper sequence of closure of that sphincter might cause bile to be forced into the gall bladder. If this were the case it would be reasonable to assume that the most effective type of closure of the sphincter would produce the greatest concentrations of bile and thus occur under the effects of the oil meal. Such closure would interfere with the entrance into the duodenum of pancreatic juice, thereby diminishing its amount and producing conditions of relative dilution of its enzymes. However, since the greatest concentrations of pancreatic enzymes followed the administrations of oil it is reasonable to assume that no such hypothecated action of the sphincter of Oddi occurred. The present authors have made numerous x-ray observations on the filling and emptying of the gall bladder after administration of tetraiodophenolphthalein, as commonly employed in cholecystography. The results merely confirm those of various other investigators (29), (30). A gall bladder filled with this dye under proper conditions shows a dense shadow on the x-ray film. About two hours after the subject has ingested 50 cc. of cottonseed oil the shadow will have diminished in size from 50 per cent to 75 per cent; the density of its shadow will either remain the same or increase. Sosman, Whitaker and Edson (31) found it took the gall bladder from 3 to 6 hours to empty after a meal rich in fats. These findings show that the gall bladder evacuates its contents slowly. Therefore, under the conditions of the experiments outlined in chart 1 a minor portion, at most, of the gall bladder contents was present in the duodenal biles collected. Since the gall bladder does not

undergo rapid emptying and filling during digestion there remains but little opportunity for bile to rapidly enter and leave that organ on its way to the duodenum. The established interchange of bile between the gall bladder and common duct which occurs (30) transpires over a much longer period of time and under entirely different experimental conditions than those under which the observations being reported were made. If such an interchange of bile were occurring under the influence of the experimental conditions reported here the following phenomena would be anticipated. Entrance of an appreciable quantity of bile into the gall bladder would necessarily dilute its contents and would retard the shadow on the x-ray film from diminishing in size although decreasing its density. However, after food ingestion the x-ray film shows that the gall bladder shadow acts exactly in the reverse manner. Obviously, if bile from the common duct were "washing out" bile in the gall bladder the shadow on the film would decrease in density regardless of whether it diminished in size. However, such decrease in density does not occur. The author's observations confirm those of Graham (30) that if digestion is in progress no x-ray shadow of the gall bladder will be obtained after administration of the dye. The most plausible reason for this is that relaxation of the sphincter of Oddi, of the distal end of the common duct, permits bile to flow directly into the duodenum (30). These observations present evidence that little or no bile passed through the gall bladder during the periods of collection of concentrated duodenal bile reported in the experiments outlined in chart 1.

The results of the chemical analyses of bile and of cholecystography discussed above indicate that under conditions of normal digestion the concentration of duodenal bile is influenced more by the functional activity of the liver than of the gall bladder.

As was discussed not infrequently relatively large volumes of fasting duodenal contents were collected. Also, collections were made the period following the stimulating effect of the various administered substances in seven experiments (chart 1). Careful studies of the figures for pepsin and chloride concentrations in these or in the fasting specimens show that such concentrations were as great as during the period of stimulation of concentrated bile. These findings indicate that relatively as much gastric juice was present in the absence of bile stimulation as in its presence. Obviously, therefore, the acidity of gastric juice was not the essential factor causing the secretion of concentrated bile. Nevertheless, it is true that N/40 HCl introduced directly into the duodenum stimulated the flow of relatively concentrated bile. However, the chemically inert $MgSO_4$ solution acted likewise. In animals it has been proved that a solution of magnesium sulphate exerts a well marked cholagogic effect when the proper concentration enters the liver directly through the portal circulation (15).

These observations furnish an explanation for the stimulating effect of magnesium sulphate noted in the experiments reported here on the basis that the proper degrees of concentration reached the liver. They also offer a similar explanation for the action of the other inorganic substance, HCl, which reached the liver as NaCl. Therefore, the flow of concentrated bile produced by the $MgSO_4$ and HCl solutions can be adequately explained as the result of direct stimulation of the liver cells by the inorganic substances $MgSO_4$ and NaCl.

Dextrose produced stimulation in experiment 8, chart 1, none in experiment 9_a chart 1, and in view of the concentration of the fasting bile little or none in experiment 7, chart 1. Peptone produced stimulation for one half a period in experiment 4, chart 1, and for a period in experiments 5 and 6, chart 1; although in experiment 6 the peak of stimulation persisted only a one-half period. Oil was a more powerful stimulant than dextrose or peptone (chart 1 and diagrams 1, 2, 3, 4). Clearly, therefore, the secretion of bile reacts differently to different types of foodstuffs. In view of the conclusion that duodenal bile comes largely directly from the liver, these findings indicate that its flow resulted from secretory activity of the liver stimulated by the foodstuffs introduced.

Whipple (25) and Pfaff and Balch (3) have shown that bile acids are cholagogues. Mann (32) proposes the theory that during digestion the acid chyle from the stomach causes the sphincter of Oddi to relax which allows bile to enter the duodenum. As has been reported (1) the gastric chyle is neutralized before it reaches the region of that sphincter. Secondly, if Mann's theory is correct that bile acids are the normal cholagogues, what would ever permit the flow of concentrated bile to cease after having once begun? On the other hand, if the foodstuffs and inorganic substances used in the present report were the direct stimulants to the flow of concentrated bile the cessation of the latter after their absorption is readily comprehensible.

The effects of the intraduodenal administration of pure foodstuffs and of HCl and $MgSO_4$ solutions on the concentrations of pancreatic enzymes, gastric pepsin and chlorides of the specimens of duodenal contents composing chart 1 were investigated. One purpose of this study was to ascertain whether the concentration of any one fraction of duodenal contents was the result of actual or relative dilution with the other fractions. The comparative results of the chemical analyses of the three fractions in each specimen of duodenal contents showed that the degrees of concentration of all of them coincided. This observation indicates that increased concentrations were the result of true organ stimulation and that lack of the same was not due to dilution by the other fractions.

This study furnished data concerning the factors involved in stimulating external pancreatic secretion. As previously stated the periods during

which collected duodenal contents are representative of the effects of stimulation of the flow of pancreatic juice were established in various reported studies (1), (4). For this reason, the present discussion may be limited to the findings in fasting contents and in those showing the stimulative effects of the administered substances, all of which are outlined in chart 1. An adequate conception of the results may be gained from the study of the minimum and maximum analytical figures which are outlined in the table which follows. The table includes the results of fifteen fasting duodenal

TABLE 4

Minimum and maximum concentrations of enzymes and chlorides of duodenal contents before and after intraduodenal administration of cottonseed oil, beef peptone, dextrose, HCl and $MgSO_4$

SUBJECT	EXPERIMENT NO.	TEST MEAL	PANCREATIC ENZYME CONCENTRATIONS						PEPSIN CONCENTRATION		CHLORIDE CONCENTRATION	
			Amylase		Lipase		Trypsin					
			Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
H	1		0.1	3.8	0.5	0.8	0.9	12.0	0.8	12.9	5.6	6.8
R	2		1.9	3.6	0.2	1.2	1.3	4.2	0.7	1.0	5.9	6.4
A	3		0.9	1.9	0.1	0.4	2.2	2.7	0.3	2.2	4.7	6.4
H	4	Oil	3.0		1.6		7.7		0.8	0.9	3.4	4.2
R	5	Oil	4.0	4.5	1.2	1.5	5.7	8.6	0.6	1.0	5.2	7.0
A	6	Oil	4.7	4.8	1.0	2.3	5.4	9.2	2.6	2.9	4.2	5.0
H	7	Peptone	2.2		1.4		5.2		5.2	15.7	7.0	7.6
R	8	Peptone	3.6		1.0		9.5		2.0		8.5	
A	9	Peptone	1.0		1.1		3.2		2.2	9.6	5.3	6.8
H	10	Dextrose	0.9	2.4	0.2	0.3	2.8	5.1	0.5	1.0	3.4	4.8
R	11	Dextrose	0.7		0.5		3.3		1.6		4.1	
A	12	Dextrose	0.2	0.5	0.2	0.3	1.1	1.3	0.6	2.1	2.7	3.5
H	13	HCl	1.0	2.0	1.1	1.2	2.8	3.5	0.7	0.8	3.2	6.4
R	14	HCl	0.7		0.8		3.8		0.7		4.2	
A	15	HCl	0.9	1.9	0.5	0.8	3.2	4.1	0.2	0.3	3.6	8.0
H	16	$MgSO_4$	1.2	1.5	1.1	1.3	3.7	4.1	1.0	1.1	6.4	7.0
R	17	$MgSO_4$	0.9	1.4	0.8	1.0	3.9	4.6	0.9	1.1	5.2	7.0
A	18	$MgSO_4$	1.4	1.5	0.9	1.0	4.4	4.4	0.7	0.8	4.4	4.6

contents; five from each normal subject taken preliminary to administration of the various substances. After the introduction of the latter 37 specimens of duodenal contents were collected and analysed.

Table 4 shows that peptone administration was followed by well marked increases in pepsin concentration of duodenal contents. Otherwise pepsin concentration was comparable in all experiments including those specimens collected during the fasting state. Chloride concentration was increased after peptone administration. It was the least after introduction of HCl and dextrose although the differences from $MgSO_4$ and oil were not great.

These findings furnish evidence of stimulation of gastric juice by peptone only.

The pancreatic enzymes of fasting contents (table 4) vary from relatively small to large concentrations. Here, as in previous observations (1), (4), the stimulation of the external enzymic function of the pancreas by foodstuffs or inorganic salts is characterized by the substantially greater minimum concentrations of the enzymes and by lesser degrees of difference between the minimum and maximum figures. Oil administration was followed by the greatest concentration of pancreatic enzymes of all substances, pepsin the next, magnesium sulphate more than HCl solution and dextrose the least. The findings show that dextrose administration produced no stimulation while it occurred only once (expt. 10, chart 1) in the three HCl experiments. This interpretation is substantiated by comparison of the analytical figures for the fasting contents obtained immediately prior to, with those following the administration of dextrose (expts. 7, 8, 9, chart 1) and HCl solutions (expts. 10, 11, 12, chart 1). The failure of HCl to stimulate the flow of pancreatic juice has been previously reported (33). These findings confirm previous observations (1), (4) that fat is the most efficient pancreatic enzymic stimulant and that protein digestion products are of next order, which indicates that they exerted a selective influence on the enzymic function of the pancreas.

The findings discussed above show that the concentration of pancreatic enzymes secreted was influenced by the type of stimulant administered. These findings do not agree with those of Ivy (34) obtained from the study of the secretion of pancreatic transplants in dogs. However, the transplants were undergoing atrophy and for this reason whether they functioned normally is debatable. Furthermore, most unfortunately the methods used by Ivy for estimating enzymic concentration are grossly inexact. For these reasons Ivy's findings are not comparable with those reported for the investigation of the present authors.

In general the concentrations of pepsin and chlorides during the periods of the dextrose experiments (expts. 7 and 9, chart 1) which produced no pancreatic stimulation were comparable to those of the $MgSO_4$ experiments and to the oil experiments (expts. 1, 3, 13 and 15, chart 1) in which pancreatic stimulation occurred. In five experiments (expts. 2, 3, 4, 10, 13, chart 1) the amounts of fasting contents recovered and the concentrations of pepsin and chlorides were as great as were present after stimulation had occurred following the administration of oil, peptone, $MgSO_4$ or HCl. These observations indicate that the volumes and concentration of gastric juice entering the duodenum were comparable both before and after the pancreas was stimulated. The inert chemical substance, $MgSO_4$, produced more pancreatic stimulation than did HCl solution. Indeed, the administered HCl produced no demonstrable stimulation in experiments

11 and 12, chart 1. These findings show that something other than the presence of HCl or the introduction of acid gastric contents into the duodenum is necessary in order to stimulate the external enzymic function of the pancreas to a degree equalling that following the administration of fat or protein foodstuffs or indeed the inert inorganic salt, $MgSO_4$. These findings confirm the interpretation made of the results of previously reported observations (1), (4) in some of which it was shown that the acid of gastric juice is neutralized in the region of the pyloric sphincter; indeed, these observations indicated that it is highly probable that no more than traces of HCl ever normally bathe the walls of the duodenum. Obviously, the interpretation made of the experimental results discussed show that it is problematical whether the acid of gastric juice is in any way concerned in the secretion of pancreatic enzymes. In other words, the evidence as interpreted does not support the usual hormonal theory proposed for the stimulation of the secretion of pancreatic juice. Similar conclusions were drawn from previous observations (1), (4). These observations are more indicative of direct stimulating effect of the absorbed substances on the pancreas than of hormonal action, a conclusion also drawn from previous observations (1), (4).

SUMMARY AND CONCLUSIONS

Procedures have been developed which permit the collection of those contents from the duodenum of man which are representative of the effects of administration of various substances on the character of bile, pancreatic and gastric juices entering this region. Methods have been developed for the quantitation of the constituents of these entities. The data obtained afford indices to the degrees of activity of various secretory functions of the liver, pancreas and stomach. Using these procedures and analytical methods the effects on the activities of these various functions of pure foodstuffs, and of solutions of HCl and $MgSO_4$ have been studied. The possibility of the volume of one fraction masking the effects of the other fractions through dilution has apparently been eliminated because of the observation that increases or decreases in the analytical figures rarely failed to coincide.

The findings show that cottonseed oil is the most powerful stimulant to the secretion of concentrated bile; peptone and the inorganic compounds HCl and $MgSO_4$ occupying second place, while dextrose was neutral in its stimulative effects. These findings confirm those previously reported (1), (4). HCl was a somewhat less potent stimulant to the secretion of concentrated bile than was $MgSO_4$. HCl was a much less potent stimulant to the flow of concentrated pancreatic juice than was $MgSO_4$. Otherwise the character of the pancreatic fraction was affected by all substances administered in a manner comparable to the bile fraction. Gastric con-

tents had no demonstrable effect on the secretion of concentrated bile or pancreatic juice. These findings are interpreted as showing that the three following phenomena occur during intestinal digestion in man.

First, they show that the concentrations of bile and of pancreatic juice present in the duodenum vary with the type of stimulant administered to the subject.

Second, they, as did previous observations, indicate that the acid of gastric contents is not the essential factor in stimulating the secretion of concentrated bile or pancreatic juice. The findings contradict the hormonal, secretin theory as usually explained.

Third, apparently cottonseed oil and peptone stimulate the liver and pancreas through secretagogic influences. The stimulating effects of HCl and $MgSO_4$ on the liver may be explained as a result of a proper concentration reaching it through the portal circulation; a comparable phenomenon may possibly explain the effect on the pancreas of the $MgSO_4$ and the occasional stimulation following administration of HCl.

The findings show that the flow of concentrated bile ceases at a time when bile salts are present in the duodenum. This observation and the others previously discussed contradict the theory that bile acids are the usual stimulants maintaining the flow of bile during intestinal digestion.

Observations were made on the varying proportional relations of the solid bile constituents and on the bromine numbers of the fatty acids found in successive specimens of the bile of normal subjects and of patients whose gall bladders had been removed, and also on x-ray pictures of the gall bladder after filling it with radiopaque dye. The results of these observations indicate that during intestinal digestion the concentrations of solid constituents of duodenal bile are more the result of functional activities of the liver than of the gall bladder. That is, duodenal bile is probably largely derived directly from the liver.

No explanation is offered for the persistent findings of bile, pancreatic and gastric juices in the duodenum during the fasting state.

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CHANGES IN THE VISCOSITY OF THE BLOOD IN NORMAL, SPLENECTOMIZED AND ADRENALECTOMIZED ANIMALS FOLLOWING EMOTIONAL EXCITEMENT

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The viscosity of the blood was reported by Burton-Opitz (1900) to be proportional to the number of red blood corpuscles per unit volume. Bugnard (1929) found a rise in the viscosity during the first forty-eight hours after splenectomy in dogs with a subsequent return to normal. Viale and Bruno (1927) observed an increased viscosity of the blood in suprarenal insufficiency in dogs, attributing this condition to a loss of plasma caused by vascular permeability. This observation was confirmed by Swingle and co-workers (1933).

Polycythemia after emotional excitement has been found by a number of investigators among them Lamson (1915–1920) with cats; Izquierdo and Cannon (1928) also with cats; and Nice, Morris and Elhardt (1930) with rats.

A decided increase in the blood sugar after emotional excitement has been observed by many workers, for example Scott (1914) with cats, Hirsh and Reinbach (1913) with rabbits and (1914) with dogs and Lumley and Nice (1930) with rats.

A concentration of most of the other chemical elements in the blood in emotional excitement has been reported by Katz and Nice (1933).

METHODS. In this investigation male albino rats and a few rabbits were used. The rats ranging from 60 to 100 days in age, were lightly anesthetized with ether and approximately one-third of a cubic centimeter of blood was drawn directly from the heart by means of a 2 cc. Luer syringe. The viscosity of the blood was measured at 26°C. by means of a Hess viscosimeter (Bircher, 1921). A stable temperature is necessary during these determinations since an inverse relationship has been found to exist between temperature and viscosity (Burton-Opitz, 1900; Snyder and Todd, 1911).

After this determination the rats were allowed to recover from the light anesthesia. They were then teased by being stimulated with a weak Faradic current from time to time for three minutes. Blood was again drawn as above from the heart and the viscosity measured. The second

sample of blood was obtained about thirty minutes after the first. By this method sometimes venous blood, but more often arterial blood, was obtained.

In the experiments with the rabbits no anesthesia was used.

Each figure in the following tables is the average of 3 to 6 viscosity determinations.

TABLE 1

Changes in the viscosity of the blood in 24 normal rats

TYPE OF BLOOD	QUIET STATE	TYPE OF BLOOD	EXCITED STATE	PER CENT INCREASE
A*	3.29	A	3.90	18.5
A	3.91	A	5.25	34.3
A	4.64	A	4.75	2.4
A	3.55	A	4.19	18.0
A	3.67	A	5.06	37.9
A	4.18	A	5.55	32.8
A	3.74	A	5.02	34.2
A	4.67	A	5.14	10.1
A	3.03	V	4.64	53.1
A	4.61	V	5.10	10.6
A	3.84	V	5.03	30.9
V*	4.20	V	6.15	46.4
V	3.81	V	4.64	21.8
V	4.15	V	5.81	40.0
V	4.40	V	5.32	20.9
V	4.68	A	5.10	8.9
V	4.50	A	5.20	15.5
V	4.65	A	5.03	8.2
V	4.36	A	5.28	21.1
V	4.46	A	4.83	8.3
V	4.36	A	5.18	18.8
V	4.53	A	5.00	10.4
V	4.70	A	5.67	20.6
V	4.33	A	5.30	22.4
Average.....	4.18		5.09	21.8

*A = Arterial; V = Venous.

RESULTS. In the normal rats as seen in table 1 there was a marked increase in the viscosity of the blood following emotional excitement (fear, pain). The average figure for twenty-four rats in the quiet state was 4.18 and after excitation 5.09, which is an increase of 21.8 per cent. Every one of the animals gave an increase.

With the rabbits in table 2 we find a somewhat lower viscosity both in the quiet and excited states than with the rats; this is to be expected since the rabbit has fewer red blood corpuscles per cubic millimeter of blood than

TABLE 2-A

Changes in the viscosity of the blood in 12 normal rabbits

TYPE OF BLOOD	QUIET STATE	TYPE OF BLOOD	EXCITED STATE	PER CENT INCREASE
A	3.39	A	4.17	23.0
A	3.37	A	4.07	20.8
A	3.35	A	4.04	20.6
V	3.50	A	3.70	5.7
V	3.50	A	3.73	6.6
V	3.92	A	4.28	9.2
V	3.53	A	3.69	4.5
Average.....	3.51		3.95	12.5

TABLE 2-B

Controls

TYPE OF BLOOD	QUIET STATE	TYPE OF BLOOD	QUIET STATE	PER CENT INCREASE
A	3.13	A	3.25	3.8
A	3.53	A	3.53	0.0
V	3.54	A	3.47	−1.9
V	4.80	V	4.77	−0.6
V	3.92	V	3.96	1.0
Average.....	3.78		3.80	0.5

TABLE 3

Changes in the viscosity of the blood in 16 adrenalectomized rats

DAYS AFTER OPERATION	TYPE OF BLOOD	QUIET STATE	TYPE OF BLOOD	EXCITED STATE	PER CENT INCREASE
4	A	3.45	A	3.76	8.9
4	A	4.26	A	5.89	38.3
4	A	4.71	A	6.65	41.2
3	A	3.14	A	3.35	6.7
3	A	3.45	A	4.07	17.9
3	A	5.01	A	6.92	38.1
5	A	4.43	A	5.25	18.5
5	A	4.91	A	5.74	16.9
5	A	4.22	A	5.50	30.3
3	A	4.83	V	5.35	10.8
5	A	3.55	V	4.33	21.9
4	V	3.99	A	5.21	30.6
3	V	4.40	A	4.95	12.5
3	V	4.43	A	4.93	11.3
4	V	4.35	V	6.88	58.2
3	V	4.80	V	5.26	9.6
Average.....		4.24		5.25	23.82

the rat. It is possible also that the use of ether slightly increased the viscosity in the blood of the rats.

TABLE 4

Changes in the viscosity of the blood in 29 splenectomized rats

DAYS AFTER OPERATION	TYPE OF BLOOD	QUIET STATE	TYPE OF BLOOD	EXCITED STATE	PER CENT INCREASE
2	A	4.65	A	4.48	−3.7
2	A	4.55	A	5.35	17.6
2	A	3.75	A	3.93	4.6
2	A	2.55	A	3.10	21.6
2	A	3.99	A	5.05	26.6
2	A	4.70	A	5.60	19.1
2	A	4.60	A	5.26	14.4
2	A	4.73	V	5.91	24.9
2	A	4.55	V	5.35	17.6
2	A	4.66	V	5.90	26.6
2	V	3.50	V	3.70	5.7
Average		4.20		4.88	15.9
3	A	3.71	A	4.28	15.1
3	A	3.42	A	3.71	8.5
3	A	4.04	V	5.05	25.0
3	A	3.93	V	4.49	14.3
Average		3.78		4.38	15.7
4	A	4.43	A	4.25	−4.1
4	A	2.41	A	2.60	7.9
4	A	2.48	A	2.41	−2.8
4	A	3.75	A	3.62	−3.4
4	A	3.99	A	4.41	10.6
4	A	3.38	A	3.75	10.9
4	V	3.91	A	3.41	−14.1
4	V	4.65	V	4.63	−0.4
4	V	3.47	V	3.60	3.7
4	V	3.92	V	4.05	3.3
Average		3.65		3.67	0.54
14	A	3.66	A	3.61	−1.4
14	A	3.61	A	3.88	7.5
14	A	3.90	A	4.10	5.4
14	V	3.98	A	3.70	−7.0
Average		3.79		3.82	0.79

The viscosity was found to be 3.51 before excitation and 3.95 afterwards, an increase of 12.5 per cent. The controls, that is, animals from which two samples of blood were taken in the quiet state, failed to show an increase.

The adrenalectomized rats in table 3 show an increase in the viscosity quite similar to that of the normal rats, the average increase was 23.82 per cent. The average values of the viscosity both in the quiet, 4.24, and excited state, 5.25, were slightly higher than that in the normal animals.

The results on our splenectomized rats in table 4 show that two days after the operation the average viscosity for the quiet blood was 4.2, which is a little higher than for our normal animals, while after excitement it rose to 4.88, an increase of 15.9 per cent. Three days after the operation the blood in the quiet state was lighter, viz., 3.78. After excitement it rose to 4.38, an increase of 15 per cent. Tests made four and fourteen days after splenectomy show no average increase after excitement, a loss being found as often as a gain.

Discussion. It was found impossible to obtain arterial or venous blood at will by the heart puncture method. In our experiments we drew the former nearly twice as often as the latter. This is to be expected because of the relation of the left ventricle to the chest wall.

TABLE 5

Summary of changes in the viscosity of arterial blood in rats

	DAYS AFTER OPERATION	NUMBER OF RATS	QUIET STATE	NUMBER OF RATS	EXCITED STATE	PER CENT OF INCREASE
Control		11	3.77	17	5.03	33.4
Adrenalectomized	3-5	11	4.18	12	5.19	24.1
	2	10	4.27	6	4.68	9.8
Splenectomized	3	4	3.78	2	4.00	5.8
	4	6	3.41	7	3.21	−0.6
	14	3	3.72	4	3.82	0.3

The average viscosity of arterial blood in control rats in the quiet state was 3.77 and of venous blood 4.32. In rabbits analogous figures stood at 3.37 and 3.61. Venous blood is considered to be more viscous than arterial because of the greater carbon dioxide content (Adam, 1909).

It is evident that when comparing venous blood in the quiet state with arterial blood in the excited state the average increase would be lower than when the same kinds of blood are compared and especially when arterial blood in the quiet state is compared with venous blood in the excited state. There are examples of all such cases in all the tables except table 2.

With the rabbits it happened that there were no examples of venous blood drawn in the excited state. Hence, the percentage of increase in viscosity found is unduly low.

If the samples in the three tables on rats are totalled, we find in the quiet state forty-five examples of arterial blood and twenty-four of venous, and in the excited state forty-nine of arterial and twenty of venous. However,

the proportions of the two types of blood, vary greatly in each division, thus masking the true differences. To obtain a clearer picture we will consider the cases of arterial blood only and these are summarized in table 5.

Here we find that both adrenalectomized and the recently splenectomized rats show marked increases in viscosity in the quiet state in comparison with the controls. The per cent of increase in the excited state is greatest in the controls, dropping somewhat with the adrenalectomized animals and markedly with the splenectomized rats. With this last group the viscosity of the blood shows a marked drop three and four days after operation in the quiet state, and a progressive disappearance of increased viscosity after excitement. The three animals tested fourteen days after operation show a return to the normal level in the quiet state, but this may have been due to chance because of the small number of subjects.

The greater viscosity of adrenalectomized and recently splenectomized animals in comparison to controls is in keeping with our previous work (Nice, Morris and Elhardt) in which we found a concentration of red blood corpuscles in the first two categories of subjects. It also agrees with the findings of the other investigators mentioned above. This concentration may be due to the animals' taking an insufficient amount of water after the operation or to a disturbance of the normal osmotic pressure of the body fluids.

SUMMARY

Venous blood had a higher viscosity than arterial, the two types averaging 4.32 and 3.77 respectively in our normal rats. In the rabbits the former averaged 3.61, the latter 3.37.

The viscosity of the blood of the normal animals was increased 21.8 per cent following emotional excitement in the rats and 12.5 per cent with the rabbits.

The viscosity of the blood in adrenalectomized rats was higher than in the controls, both in the quiet and emotionally excited states.

In the splenectomized rats two days after the operation the viscosity was higher than normal in the quiet state, but the increase after emotional excitement was less than with normal rats, viz., 15 per cent. After four days the viscosity decreased markedly both in the quiet and excited states.

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THE DETERMINATION OF THE PLASMA VOLUME AND THE BLOOD VOLUME OF THE RABBIT BY THE INJECTION OF HOMOLOGOUS ANTI-CRYSTALLIZED-EGG-ALBUMIN-SERUM

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In experimental studies upon animals, it is often necessary that the plasma volume or the total blood volume be known accurately. Numerous methods for the estimation of these volumes have been proposed.[1] In some, the amount of blood is found directly by bleeding the animal and washing out its blood vessels. The other procedures fall under the general head of indirect methods, since they involve the addition of a known amount of some material to the blood and the subsequent determination of the dilution of the substance or of the blood. The considerable variation seen in the figures for plasma volume or total blood volume by the indirect procedures and the somewhat higher values usually obtained by these methods compared with the direct methods may be due partly to loss of the injected foreign material from the blood stream and partly to the difficulty in determining the dilution of the substance introduced.

An immunologically distinct blood or serum would, presumably, be more compatible with the blood and tissues of an experimental animal than a dye or other foreign chemical substance. The dilution of such a blood can be determined by immunologic tests. The first to employ an immunologic technic were Dreyer and Ray (1909), who injected an agglutinating serum and determined its dilution in the blood after 24 hours. Schurer (1911) injected a heterologous normal serum and by means of the precipitin reaction with a potent antiserum found its dilution and, thus, the plasma volume. The procedure of von Behring (1911) consisted in finding the dilution of a known amount of tetanus antitoxic serum after its introduction into the blood stream.[2] Todd and White (1912) employed isohemolysins to determine the dilution of blood from another animal of the same species after its injection. Ashby (1920, 1925) described a similar method which made use of isohemagglutinins for finding the blood volume of man.

[1] For a review of methods, consult Erlanger (1921).

[2] This procedure was described by Ehrlich and Lazarus (1901).

These immunological methods have not been widely used, although they would seem potentially advantageous since a native animal substance is administered. They have been neglected in part perhaps because the immunologic methods employed in determining the dilution of the injected blood or serum lack sufficient precision. We have described a method for the quantitative estimation of the precipitin antibody in rabbit anti-crystallized-egg-albumin-serum (Culbertson, 1932) which appears to satisfy the requirement of accuracy and have applied this procedure to the determination of the blood plasma volume of rabbits.

THE METHOD FOR DETERMINING PLASMA VOLUME BY THE INJECTION OF A HOMOLOGOUS ANTISERUM. A known volume of rabbit anti-crystallized-egg-albumin-serum rich in precipitin is injected into the marginal ear vein of a normal rabbit (not anesthetized) immediately after the same amount of blood has been drawn from the recipient's heart. At 5, 15, and 60 minutes and 24 hours after the injection, 3 cc. blood samples are taken from an ear artery of the injected animal. By means of the "neutralization method" for estimating precipitin, the precipitin content is determined for the injected serum and for the serum of the blood samples taken from the recipient rabbit after it has received the antiserum. The plasma volume of the injected rabbit is found by the formula:

$$\text{Blood plasma volume} = \frac{\text{mgm. precipitin per cc. of injected serum} \times \text{cc. of antiserum injected}}{\text{mgm. precipitin per cc. of recipient's serum post injection}}$$

The estimates of plasma volume must be corrected to account for the difference in the volumes of the plasma withdrawn and the serum injected. This difference, which equals the volume of the cells removed, is subtracted from the value given by the formula above. The volume of the cells removed is determined by finding the ratio of cells to plasma in an hematocrit determination of the blood of the injected rabbit.

The "neutralization method" for estimating the precipitin in the serum. The precipitin of the injected serum and of the serum of the blood samples withdrawn after injection were determined by a procedure called the "neutralization method" which we have described at length in an earlier paper (Culbertson, 1932). This requires but little serum and thus permits that the animal be bled at short intervals of sufficient blood for quantitative estimates of the antibody. The method consists essentially in determining that amount of antigen which precipitates apparently all the precipitin from the antiserum—that is, in determining the "neutralization point," where, within the limits of delicacy of the method, neither antigen nor antibody is detectable in the supernatant fluid after precipitation has taken place.

The stock solution of crystallized egg albumin is diluted with saline to provide known concentrations of the antigen expressed in milligrams per cubic centimeter. One-tenth cubic centimeter of each antigen dilution is mixed with an equal volume of the antiserum tested, incubated 2 hours at 37 degrees, then put in the refrigerator overnight. On the next day, after centrifugation, the supernatant fluids over the precipitates are tested for antigen and for antibody. To test for antigen, half the supernatant fluid of each tube is layered over an equal volume of known strong antiserum in a small precipitin tube; to test for antibody the remaining half of the supernatant fluid of each tube is put into a second tube and overlayed with an equal volume of the antigen diluted 1–20,000. These tubes are incubated 1 hour at 37°C., read for "rings," incubated a second hour after the fluids are mixed, and put in the cold overnight. On the next day, the tubes are read for precipitation. If suitable dilutions of antigen have been employed, the supernatant fluid of one tube will be found to contain neither antigen nor antibody within the limits of delicacy of the test, whereas other tubes will contain either the antigen or the antibody, depending upon which was present in excess in the initial mixture. The mixture in the tube whose supernatant fluid contains neither substance is said to be at the neutralization point.

A large number of tests have shown that the antigen unites with the antibody in a constant ratio in terms of nitrogen (about 1:13) at the "neutralization point"; hence, when the amount of antigen required to establish this point is known, the amount of precipitin present in the serum can be calculated. A considerable experience with the method indicates for it a limit of error of about 10 per cent.

The method appears useful chiefly for estimating precipitin quantitatively when only small amounts of antiserum are available and when this is of relatively low potency.

The preparation of potent anti-crystallized-egg-albumin-serum. The potent anti-crystallized-egg-albumin-serum for the transfusions was prepared by injecting rabbits intravenously with the antigen according to the following schedule: 1.25 mgm. protein per kilogram body weight 5 times weekly for 2 weeks, and 2.5 mgm. protein per kilogram body weight 5 times in the third week. The animals were bled between the fourth and sixth day after the last injection. If a preliminary test showed that the serum was still low in precipitin, the injections were continued another week. Occasionally a rabbit was encountered which failed to give a good yield in the antibody even after a protracted immunization period. It was found best to substitute others for those which did not respond promptly.

The total amount of antigen for immunization (25 mgm. protein per kilogram of body weight) although less than that employed by earlier workers with this antigen was sufficient for the purpose. Indeed, it seems likely that fractions of this quantity would do, perhaps as well. The better antiserums which we have prepared by this procedure have contained on the average about 5.0 mgm. antibody protein per cubic centimeter. The strongest serum yet produced contained over two times this amount of precipitin (10.96 mgm. antibody protein per cc.).

The test for normal precipitin against crystallized egg albumin. Although the normal rabbit only very rarely, if ever, possesses precipitin for crystallized egg albumin, it is essential that the test be made upon all animals whose plasma volume is to be determined. The serum from 1 cc. or less of blood from each animal suffices for this purpose. A small amount of each serum (0.1 cc.) is put into a small-bore precipitin

tube and overlayered with an equal quantity of a 1–20,000 dilution of the egg albumin. The antigen must be used in the high dilution, since concentrated antigen solutions inhibit the reaction when only a small amount of antibody is present. The tubes are incubated at 37°C., for one hour, then examined for "ring" formation at the interface of the two fluids. The fluids are mixed, incubated a second hour, then placed in the cold overnight. On the next day, the tubes are examined for precipitates. Animals which possess normal precipitin for egg albumin should never be used for plasma volume determinations by the method here offered.

TABLE 1

The data obtained upon the volume of the blood plasma and the whole blood of rabbits by injecting rabbit anti-crystallized-egg-albumin-serum

RABBIT NUMBER	SEX*	WEIGHT	ANTISERUM INJECTED	PRECIPITIN INJECTED	PRECIPITIN IN PLASMA POST-INJECTION (MGM./CC.)				PLASMA VOLUME (15 MIN.)	PERCENTAGE PLASMA IN WHOLE BLOOD	PERCENTAGE OF BODY WEIGHT	
					5 min.	15 min.	60 min.	1 day			Plasma volume	Blood volume
		grams	*cc.*	*mgm./cc.*					*cc.*			
1	♂	2025	20	5.28	1.41	1.41	1.31	0.60	64.6	51	3.19	6.25
2	♂	2070	20	9.34	2.43	2.43	2.43	1.51	67.4	54	3.25	6.01
3	♂	2130	15	4.46	1.01	1.01	0.91	0.60	59.4	55	2.79	5.07
4	♂	2260	15	7.31	1.51	1.31	0.91	0.60	75.0	50	3.31	6.62
5	♂	2290	20	7.31	1.03	1.51	1.21	1.01	87.7	58	3.82	6.10
6	♂	2290	20	3.5	0.70	0.70	0.70	0.40	84.0	60	3.66	6.10
7	♂	2390	20	5.28	1.21	1.21	1.01	0.81	77.8	56	3.25	6.58
8	♂	2540	20	4.46	1.01	1.01			78.4	51	3.08	6.03
9	♂	3100	20	4.87	1.01	0.81	0.81	0.40	111.0	55	3.58	6.50
10	♂	3435	17	5.28	0.81	0.81	0.81	0.40	103.7	60	3.01	5.01
11	♀	1820	15	3.65	1.10	0.91	0.91	0.40	53.3	55	2.92	5.31
12	♀	1935	18	4.06	1.15	1.01	0.91	0.60	63.1	50	3.26	6.52
13	♀	2255	15	6.50	1.15	1.10	1.01	0.81	82.6	63	3.66	5.95
14	♀	2400	20	6.50	1.41	1.31	1.31	0.60	89.6	53	3.73	7.03
15	♀	2990	26	5.28	1.31	1.31	1.31	1.21	93.1	56	3.11	5.55
16	♀	3410	20	2.78	0.70	0.60	0.60		84.8	55	2.48	4.50
17	♂	2080	15	5.62	1.33	1.33	1.18	0.58	56.7	**	2.72	4.85
18	♂	2130	10	6.25	0.74	0.74	0.74	0.44	79.6	**	3.73	6.66
19	♂	2230	15	8.12	1.33	1.47	1.33	0.89	75.9	**	3.40	6.07
20	♂	2480	15	2.93		0.52			77.2	**	3.11	5.55
21	♂	2540	15	6.50	1.18	1.18	0.89	0.81	75.9	**	2.87	5.12
22	♀	1760	12	6.25	1.03	1.03	0.89	0.58	66.7	**	3.78	6.75
23	♀	2070	15	3.25	0.58	0.74	0.58	0.30	58.9	**	2.84	5.07
24	♀	3040	15	3.81	0.66	0.66	0.66	0.44	78.9	**	2.59	4.62
Average										55	3.21	5.82

* No female was pregnant.

** Hematocrit determinations of cell-plasma ratio were not made; plasma percentage taken as 55, the average of first 16 animals.

THE RESULTS OF THE APPLICATION OF THE PROPOSED METHOD. The data are presented in table 1 upon 24 rabbits whose plasma volumes were

found by the method described. The average plasma volume in cubic centimeters is found to equal 3.21 per cent of the figure for the weight in grams of the animals. Since, according to the hematocrit determinations given, the plasma makes up 55 per cent of the whole blood, the total blood volume in cubic centimeters equals 5.82 per cent of the figure which represents the weight in grams.

The determination of the precipitin of the blood samples taken 5, 15 and 60 minutes after injecting the precipitating antiserum indicates that the mixture of the antiserum and the blood is usually complete within 5 minutes and is maintained with but little change for 15 minutes. We have selected the 15 minute bleeding as the optimum rather arbitrarily. Since in 15 minutes about 60 complete circulations probably occur, there appears ample opportunity for mixing of the antiserum with at least that blood in

TABLE 2

The disappearance of the precipitin from the circulation of rabbits injected with homologous anti-crystallized-egg-albumin-serum

RABBIT NUMBER	WEIGHT	ANTISERUM INJECTED	PRECIPITIN INJECTED	PRECIPITIN IN BLOOD SAMPLES: (MGM./CC.)														
				Normal	5 minutes	15 minutes	1 hour	1 day	2 days	4 days	6 days	8 days	10 days	13 days	17 days	20 days	24 days	26 days	30 days
	grams	*cc.*	*mgm./cc.*																
1	2025	20	5.28	0	1.41	1.41	1.31	0.60	0.40	0.30	+	+	+	+	+	+	+	0	0
14	2400	20	6.50	0	1.41	1.31	1.31	0.81	0.60	0.40	0.20	+	+	+	+	+	+	+	0

active movement. Over 90 per cent of the precipitin found per cubic centimeter is still in each cubic centimeter of the plasma one hour after the injection and over 50 per cent is present 24 hours later. In two animals, traces of the antibody were found to persist in the circulation for over 3 weeks after the injection (see table 2).

Discussion. Perhaps the greatest advantage of the method presented here rests upon the probability that the injected substance is harmonious with the blood and tissues of the experimental animal. If this be true, there is likelihood of less activity on the part of the animal to eliminate this substance than distinctly foreign materials. However, the percentage reduction in concentration in the first few minutes after the injection is not greatly different whether vital red dye, gum acacia, or antiserum be injected. In samples taken after 20 minutes, an average of 91.8 per cent of the dye in the 2-minute sample is found (Hooper, Smith, Belt, and Whipple, 1920). In the case of the acacia, equal amounts (of furfurol-phloroglucid) are detected in 5 and 10-minute samples (Meek and Gasser, 1918–19). The 15-

minute samples by the method we have proposed contained an average of 96.6 per cent of the precipitin found in the 5-minute bleedings. After an interval of one hour, however, only 73 per cent of the dye remained in the blood (Keith, Rowntree, and Geraghty, 1915) while 91.2 per cent of the acacia (Meek and Gasser, 1918–19) and 90.9 per cent of the precipitin was still circulating. Only 10 to 26 per cent of the dye (Hooper et al., 1920; Keith et al., 1915) was present after 24 hours, at which time 50 per cent of the acacia (Meek and Gasser, 1918–19) and 59 per cent of the precipitin were detectable.

It is probable that much of the "loss" from the circulation during the first 15 to 20 minutes, at least, is more apparent than real, and that perfect intermixture of the injected substance and the plasma requires the greater part of this time, especially in the inactive animal. Our determinations in a few rabbits (i.e., nos. 4, 5, 11, 19, and 23) indicate that a period of 5 minutes is not always long enough to permit complete mixing in the blood stream. It seems probable that part at least of the reduction after longer intervals (i.e., 1 hour, 1 day) is due to further dilution of the injected substance in the tissue fluids and the lymph.

The more severe limitations of the precipitin-injection method are that repeated determinations at brief intervals are inadvisable, and that the method in its present state is not suited to human blood volume investigation. The facts that the injected material persists for several weeks in the circulation and that, once having received antibody, the animal is no longer "normal," dictate against repeated determinations of the plasma volume by this method. The method should not be applied to man because of the danger involved. A sufficient quantity of homologous antiserum for injection into man could probably not be obtained. The present method is, then, offered only for rabbits and, possibly, some other laboratory animals.

The percentage relationship of the blood to the body weight of the rabbit is somewhat higher by the method we offer than by the direct method of washing out the circulation. Boycott (1911–12) found 4.55 per cent by the latter technic, whereas we have arrived at the value 5.82 per cent by determining the dilution of an injected precipitating antiserum. On the other hand, the figure we present is considerably below the value 8.7 per cent obtained by Went and Drinker (1929) who employed the vital red method upon rabbits. Our estimate agrees rather closely with the results of Boycott (1911–12) who used the carbon monoxide inhalation procedure (5.36 per cent), of Meek and Gasser (1918–19) who used the acacia method (5.44 per cent), and of Schürer (1911) who determined by the precipitin test the dilution of a foreign normal serum (5.66 per cent).

Compared with determinations by various methods for some other species of animals, most of the percentage figures for calculating the blood

volume from the body weight are low for rabbits. For examples, determinations for the blood volume in the dog range between 11.91 and 7.0 per cent of the body weight (Meek and Gasser, 1918–19; Hooper et al., 1920; Powers at al., 1930; Leichsenring et al., 1932) and in the rat between 7.4 and 6.13 per cent (Chisholm, 1911; Scott and Barcroft, 1924; Cartland and Koch, 1928; Went and Drinker, 1929). In man, determinations have usually fallen between 9.3 and 6.6 per cent (Douglas, 1906; Keith, Rowntree and Geraghty, 1915; Chang and Harrop, 1928; McIntosh, 1929). Most figures for the cat, on the other hand, appear well within the range of those for the rabbit; 5.5 per cent has been reported for cats by Meek and Gasser (1918–19) and by Harris (1920). It seems possible that, as Dreyer and Walkër (1913) have concluded for the hemoglobin percentage, the blood volume percentage of the body weight also varies according to "the mode of life and muscular activity of the species."

Dreyer and Ray (1912–13) have presented evidence that the quantity of blood is a function not of the body weight but of the body surface. They have found that smaller rabbits have more blood in relation to their body weight than larger rabbits. In a critical study of the data of a number of other workers who had used various methods of blood volume estimation, Dreyer and Ray (1912–13) found that the percentage of blood diminished from 7.97 in the lightest rabbits averaging 354 grams body weight to 4.39 in the heaviest animals of 3595 grams. The data we give offer but little evidence one way or the other upon this question because the range of weights among our animals is relatively narrow (1760 grams to 3435 grams). Yet it is worthy of note that for 6 or 7 of our animals which weigh over 2500 grams, the estimated blood volume percentage is below the average for the entire number of animals.

CONCLUSION

A method is presented by which the plasma volume of the rabbit can be found. It consists in determining the dilution which a known amount of homologous precipitating antiserum undergoes after injection into the circulation of the animals.

The results obtained indicate that in the rabbit the plasma volume represents 3.21 per cent and the total blood volume represents 5.82 per cent of the body weight.

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EXCESSIVE GONAD-STIMULATING HORMONE AND SUBNORMAL AMOUNTS OF OESTRIN IN THE TOXAEMIAS OF LATE PREGNANCY[1]

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One of the most striking changes during gestation in women is the appearance in the blood and urine of large quantities of anterior-pituitary-like, gonad-stimulating hormone (prolan) and of oestrin. In normal pregnancy the gonadotropic hormone is said to be present in increasing amounts until about the second to fourth month and then to decrease moderately until term (13). Oestrin is present only in comparatively small amounts in early pregnancy and gradually increases until delivery, when tremendous quantities can be demonstrated (12). It is more and more being realized that these hormones, as well as influencing the reproductive system, are closely related to the activity of other internal secretions, so that any quantitative variation from the above profound alterations which occur throughout normal pregnancy might well be associated with a general metabolic disturbance, such as is found in the toxaemias of late pregnancy. In order to discover whether or not cases of toxaemia differ from normal as regards these hormones, quantitative determinations by the Ascheim-Zondek and Allen-Doisy methods have been made on 46 sera and 44 24-hour urines from 42 women during the last third of pregnancy, 15 of whom were normal, 22 toxaemic and 5 eclamptic. A brief preliminary report has already been submitted on 27 of these patients (1).

It was necessary to include normal pregnancy in this study for two reasons. First, biological assay is not sufficiently exact so that any two laboratories would agree perfectly in quantitative results. Individual differences in the procedures of extracting specimens, of injecting animals and of interpreting results are among the unavoidable variables. Secondly, the data on the amounts of these hormones excreted are reported usually in terms of rat units per liter of urine with no consideration of the concentration of the specimens. In the cases to be reported 24-hour volumes of urine were collected and the results expressed in rat units per 24 hours. Normally pregnant women excrete approximately the same amounts of these hormones on successive days regardless of the volume of urine. For

[1] The Mrs. William Lowell Putnam investigation of the toxaemias of pregnancy.

example, the total 24-hour urines from one pregnant woman on two successive days contained the same amounts of oestrin and prolan, although the volume was 780 cc. the first day and 4050 cc. the second. If the oestrin and prolan were expressed in terms of rat units per liter it would appear that this woman had excreted five times as much the first day as the second. Murphy (2), in a recent publication, reached the same conclusion as regards the excretion of the ovary-stimulating hormone, and also pointed out the importance of collecting urines over a given period of time if one desires truly quantitative results.

Another fairly accurate method of gauging the concentration of a given specimen is by determining the creatinine, since the 24-hour urine of an individual will always contain approximately the same amount of creatinine depending upon her weight. Although practically all of the cases to be reported were hospitalized, we have determined the urinary creatinine in order to be perfectly certain that each specimen represented a full 24-hour volume. Our results, together with those of Murphy (2), make it apparent that in the quantitative analysis of these hormones, as in any other quantitative urine analysis, either the total volume excreted over a given period of time or some other gauge of the concentration, e.g., creatinine, must be known in order to procure comparable results.

METHODS. From all patients total mixed 24-hour urines were collected and 40 cc. of blood taken without any anticoagulant. The volume of urine was measured and the serum removed from the clotted blood. All analyses were carried out upon serum rather than whole blood for two reasons: first, to avoid the use of anticoagulants and, secondly, to facilitate the preparation of extracts. Kemp and Bjergaard (14) have demonstrated that the blood oestrin is practically equally divided between the corpuscles and plasma. Most of the prolan, however, we have found to be present in the plasma or serum.

In performing Ascheim-Zondek tests for the anterior-pituitary-like hormone, both urines and sera were extracted, since these materials are often toxic to immature rats. After being prepared the extracts were never allowed to stand more than two days before injections were started, because they deteriorate quite rapidly. Unextracted urines and sera, however, can stand in the refrigerator for 2 to 6 months without any loss in potency. The exact amount of specimen to be tested, therefore, was precipitated in a centrifuge tube with 5 volumes (or more) of 95 per cent ethyl alcohol, allowed to stand overnight in the refrigerator and then centrifuged. The precipitate was washed with ether, taken up in 6 cc. of saline and injected into female rats 19 to 23 days old in 6 injections over 3 days. The ovaries were examined 96 hours after the first injection. *The test was considered positive only if corpora lutea were visible grossly.* Extracts were made and tested repeatedly until the smallest amount of

material that would give a positive result and the largest amount that would give a negative result had been determined. In general it was necessary to use 10 to 20 immature rats to establish the prolan content of each serum and of each urine.

In assaying for oestrin the Allen-Doisy technique was followed. Mature spayed female rats were employed, their response to the oestrous hormone being frequently checked by extracts of known potency. It was not necessary to extract the urines for this procedure but alkaline specimens were slightly acidified with dilute hydrochloric acid. In establishing the minimum amount of a given specimen that would produce full oestrus and the maximum amount that failed to produce oestrus, 20 to 30 rats were injected. The amounts of serum to be assayed were treated with 5 (or more) volumes of 95 per cent ethyl alcohol to precipitate the proteins and allowed to stand overnight. After being centrifuged the precipitate was washed repeatedly with alcohol and finally with ether. The combined supernatant fluids were evaporated on the steam bath and the residue taken up in saline, forming an emulsion suitable for injection. The limited quantity of blood available made it necessary to test each different amount of a given serum extract upon only 2 rats. The data on sera, therefore, are reported in terms of the actual reading, +++ signifying a full oestrous smear in one or both rats, ++ a pre-oestrous reading, etc.

Table 1 presents the results on 12 controls who were a month and a half or less from term. Nine were 3 weeks or less from term. The figures are quite consistent both for prolan and oestrin in the serum and urine. Analyses of the urines on successive days (cases 7 and 8) revealed that the 24-hour excretion of these hormones does not vary appreciably from day to day. From the figures presented in table 1 it may be concluded that a normally pregnant woman within a month and a half from term excretes no more than 1000 rat units of prolan in 24 hours and no less than 4000 rat units of oestrin. There is a fairly constant ratio between the amounts of these hormones in the urine and those in the serum. At least 1.5 cc. of serum (i.e., 66.6 r.u. per 100 cc. of serum) are required to give a positive Ascheim-Zondek test (prolan), and no more than 3 cc. are required to give oestrous smears in castrated female rats (oestrin).

Table 2 contains the results on 19 patients who had toxic symptoms without convulsions and who were a month and a half or less from term. Twelve were 3 weeks or less from term. In only 2 (cases 25 and 28) was the prolan level within normal limits, whereas the other 17 had amounts, both in urine and serum, considerably above the highest of the normals. Inspection of the table indicates that the ratio of prolan in the urine to that in the serum is not as constant as in the normals. Six of these patients excreted amounts of oestrin that are within normal limits and one (case 14) excreted considerably more. The other 10 patients, whose urinary excre-

TABLE 1

Normal pregnancy—Seven and one-half months to term

CASE	DATE OF SPECIMEN	DATE WHEN DUE	HOSPITAL DIAGNOSIS	BLOOD PRESSURE	URINARY ALBUMEN	COMMENTS	ASCHEIM-ZONDEK TESTS				ALLEN-DOISY TESTS						
							Serum		Urine		Serum					Urine	
							Positive	Negative	Positive	Negative	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	Positive	Negative
							r. u. per 100 cc.	r. u. per 100 cc.	r. u. in 24°	r. u. in 24°						r. u. in 24°	r. u. in 24°
1	3/25/32	3/31/32	9 mos. Normal	115/70	0	Age 26 para I			460	680						7,500	9,200
2	6/21/32	7/ 5/32	8½ mos. Normal	120/72	0	Age 19 para I	40.0	50.0	325	410			+++			6,800	9,000
3	6/21/32	7/ 5/32	8½ mos. Normal	100/68	0	Age 16 para I	50.0	66.6	850	980		+++				6,100	8,500
4	8/ 6/32	8/19/32	8½ mos. Normal	124/70	0	Age 31 para I	40.0	50.0	270	360		+++	+++		+++	6,000	7,200
5	8/ 6/32	8/27/32	8 mos. Normal	116/64	0	Age 18 para I	50.0	66.6	340	510			+++			6,800	8,500
6	12/16/32	12/27/32	8½ mos. Normal	116/70	0	Age 16 para I	50.0	66.6	900	1,280	+±	+±±	+++			6,000	7,200
7	3/ 3/33	3/ 6/33	9 mos. Normal	130/83	0	Age 18 para I	50.0	66.6	850	1,000	+±±	+++				7,100	9,400
	3/ 4/33	3/ 6/33	9 mos. Normal	130/83	0				840	1,000						7,100	9,400
8	3/ 3/33	3/17/33	8½ mos. Normal	126/84	0	Age 19 para I	40.0	50.0	380	570	±	+±	+++			4,200	5,400
	3/ 4/33	3/17/33	8½ mos. Normal	126/84	0				350	520						4,000	5,000
9	5/25/33	7/12/33	7½ mos. Cardiac	110/55	0	Age 22 para IV	50.0	66.6	450	600	−	+±	+++			4,200	5,000
	6/ 7/33	7/12/33	8 mos. Cardiac	110/60	0		50.0	66.6	375	500	−	+++	+++			5,000	6,000
10	3/ 8/33	4/10/33	8 mos. Normal	120/70	0	Age 33 para II			900	1,115						5,000	6,200
11	5/10/33	6/10/33	8 mos. Normal	128/94	0	Age 19 para I	66.6	100.0				±	+±±				
12	5/10/33	5/22/33	8½ mos. Normal	118/82	0	Age 20 para I	66.6	100.0				±	+±±				
Average							50.0	68.0	560	735						5,800	7,400

TABLE 2

Toxaemic pregnancy—Seven and one-half months to term

CASE	DATE OF SPECIMEN	DATE WHEN DUE	HOSPITAL DIAGNOSIS	BLOOD PRESSURE	URINARY ALBUMEN	BLOOD CHEMISTRY		COMMENTS	ASCHEIM-ZONDEK TESTS				ALLEN-DOISY TESTS						
									Serum		Urine		Serum					Urine	
						N.P.N.	Uric acid		Positive	Negative	Positive	Negative	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	Positive	Negative
						mgm. per 100 cc.	*mgm. per 100 cc.*		*r.u. per 100 cc.*	*r.u. per 100 cc.*	*r.u. in 24°*	*r.u. in 24°*						*r.u. in 24°*	*r.u. in 24°*
13	12/16/32	1/10/33	8 mos. Mild toxaemia without convulsions	128/75	S.P.T.			Age 16 para I. Oedema and occasional slight albuminuria	200	333	1,900	3,100		++±	+++			6,300	7,900
14	3/23/32	3/30/32	9 mos. Toxaemia without convulsions	148/82	T	30.0	6.2	Age 22 para I. Oedema. Follow-up: 4/10/32. Alb. S.T. B.P. 90. No milk	100	200	1,525	3,100	±	++	+++			10,000	20,000
15	5/ 3/32	5/12/32	8½ mos. Toxaemia without convulsions	150/84	L.T.	25.0	4.4	Age 24 para III. Dizziness. Toxaemia with 2 previous pregnancies	100	200	5,700	8,500		−	±	++		2,800	3,800
16	5/25/32	6/ 3/32	8½ mos. Toxaemia without convulsions	140/90	T	31.0	5.5	Age 24 para I. Oedema. Follow-up: 7/1/32. B.P. 96/64. Alb. 0	200	400	2,800	4,200			−	±	±	1,800	2,800
17	6/ 3/32	7/10/32	7½ mos. Preeclamptic toxaemia	184/100	S.T.	27.0	3.4	Age 38 para VII. Oedema. No toxaemia in previous pregnancies. Follow-up: 10/9/32. B.P. 146/80	200	333	2,700	4,500		−	±	++		3,400	4,500
18	6/ 7/32	6/ 8/32	9 mos. Toxaemia without convulsions	190/110	T	30.0	4.8	Age 35 para II. Oedema. Last pregnancy normal	100	200	1,230	1,840			−		−	310	440
19	8/29/32	9/28/32	8 mos. Toxaemia without convulsions	128/90	L.T.	25.0	4.5	Age 31 para I. Oedema and headache	1,430	3,333	13,000	26,000			−		++	1,000	2,100
20	10/13/32	10/20/32	8½ mos. Toxaemia without convulsions	150/90	S.T.	24.0	3.6	Age 24 para I. Oedema. Premature delivery in 1931	333	1,000	6,800	10,000	++	+++	+++			6,800	9,800
21	11/ 5/32	12/15/32	8 mos. Toxaemia without convulsions	150/80	S.T.	28.0	4.0	Age 41 para VIII. Oedema 11/6–11/9/32: 24,000 R.U. Progynon p.o.	200	333	1,370	1,830		−	+±			2,300	3,000
	11/10/32[a]	12/15/32		135/100	0				100	200	840	1,250		−	+±			4,700	5,800

22	12/22/32	1/17/33	8 mos. Toxaemia without convulsions	160/90	L.T.	30.0	3.8	Age 42 para VI. Oedema. Follow-up: 2/10/33 B.P. 126/70 Alb. 0	200	333	8,000	13,000		−	++			6,700	8,900
23	12/24/32	1/14/33	8½ mos. Toxaemia without convulsions	160/105	0	31.0	4.4	Age 22 para I	200	333	2,600	3,700		+	+++			5,800	8,700
24	2/ 7/33	2/21/33	8½ mos. Preeclamptic toxaemia	190	T	29.0		para II. Oedema and twitching. Miscarried at 2 mos. in last pregnancy. Follow-up: 2/21/33. Alb. S.P.T	333	500	3,100	3,900		±	+±			5,200	6,880
25	1/ 3/33	2/16/33	7½ mos. Toxaemia without convulsions	170/100	T	24.0	3.6	Age 29 para II. No toxic symptoms. First pregnancy normal. Follow-up: 2/24/33 B.P. 140/68. Alb. 0	66.6	100	800	900			−			2,100	3,175
26	1/24/33	3/ 9/33	7½ mos. Toxaemia without convulsions	164/72	0	26.0	3.9	Age 32 para II. Oedema. First pregnancy normal	200	333	1,770	3,500			−			970	1,460
27	1/26/33	2/ 8/33	8½ mos. Toxaemia without convulsions	200/130	T			Age 23 para I. Twitchings—casts in urine. Premature delivery 1/26/33	100	200			+±	+++	+++				
28	3/19/33*	4/26/33	7½ mos. Chronic nephritis	160/80	H.T.	23.0	4.5	Age 22 para II. Oedema-casts in urine. Toxic symptoms and alb. began at 2 mos. Toxic separation of placenta at 5 mos. 1 yr. ago. Follow-up: 6/15/33. Alb. H.T.	50.0	66.6	520	740	−	+++	+++			7,000	8,600
29	3/25/33	4/14/33 Delivered 3/25/33	8½ mos. Toxaemia without convulsions	150/88	T			Age 18 para I. Oedema, dizziness, headache. No casts in urine	200	333			−	+++	+++				
30	3/13/33	3/23/33	8½ mos. Toxaemia without convulsions	126/78	T			Age 35 para VII. Dizziness and spotting. Previous pregnancies normal	100	200	1,800	2,250		−	+	++		3,700	5,000
31	4/17/33	4/16/33 Delivered 4/23/33	9 mos. Toxaemia without convulsions	146/100	0	25.0	2.9	Age 24 para I. Marked oedema of face. Sudden rise in B.P. Blood and urine taken before treatment	200	333	2,900	4,200		−		±		650	1,000
								Average	250	500	3,600	6,000						3,700	5,500

* The results on these specimens were omitted from the average figures.

tion was determined, excreted amounts below normal, some being strikingly low. The sera of 12 of the 19 patients with toxaemia failed to give oestrus in 3 cc. amounts, and in 7 of these, from whom there was sufficient serum to test 4 or 5 cc. amounts, the smears still failed to show full oestrus. The sera of the other 7 were normal in their content of oestrin.

The 2 cases in table 2 whose levels of prolan were normal deserve special comment. Case 25 had abnormally low oestrin in both serum and urine, so that, although her prolan appeared normal, her prolan to oestrin ratio was high. The other (case 28) is the only one of this series definitely diagnosed as nephritic. She had had a toxic separation of the placenta with macerated fetus during the fifth month of her previous pregnancy, and had begun showing large amounts of albumin and casts at the second month of this pregnancy. A month after delivery she was still showing large amounts of albumin. She is also the only one of this series whose serum and urine levels of *both* oestrin and prolan were within normal limits. It cannot be concluded from this, however, that nephritic and toxaemic conditions may be differentiated by hormone analysis since, without more complete and longer follow-ups on the other cases, it might be argued that some of them were predominantly nephritic.

In a series of 18 women, therefore, diagnosed as toxaemic without convulsions there were none whose serum and urine levels of *both* oestrin and prolan were within the limits found in a series of 12 women normally pregnant at the same period of gestation. All but one had levels of anterior-pituitary-like hormone in serum and urine higher than normal. The oestrin in the serum and urine of all but 6 was lower than normal.

It has been established by experiments on animals that oestrin inhibits the gonad-stimulating activity of the anterior hypophysis (3, 4, 5, 6, 7, 8, 9, 10). Although the source of the excessive prolan in toxaemia has not been determined, we investigated the possible effect of large amounts of oestrin upon the quantity of prolan in one patient, case 21. Over a period of 5 days, 24,000 rat units of oestrin (Schering-Corporation "Progynon") were taken by mouth. As seen in the table, the prolan of both serum and urine decreased appreciably and the excreted oestrin was greater, but the serum level remained the same. This patient was receiving hospital treatment, so that any clinical change might not be attributed to the progynon. The reduction of prolan, however, may have been affected by the ingested oestrin, since so definite a drop would not usually be expected in the course of one week. Moreover, case 33 (table 3), under the same clinical routine but without the progynon, showed changes in the opposite direction.

Table 3 gives the results of analysis for these hormones in 5 women at about the sixth month of gestation. Two of these (cases 32 and 33) were diagnosed as toxaemic without convulsions. The other three had no toxic

TABLE 3

Normal and toxaemic pregnancy—Around six months

CASE	DATE OF SPECIMEN	DATE WHEN DUE	HOSPITAL DIAGNOSIS	BLOOD PRESSURE	URINARY ALBUMEN	BLOOD CHEMISTRY		COMMENTS	ASCHEIM-ZONDEK TESTS				ALLEN-DOISY TESTS						
									Serum		Urine		Serum					Urine	
						N.P.N.	Uric acid		Positive	Negative	Positive	Negative	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	Positive	Negative
						mgm. per 100 cc.	*mgm. per 100 cc.*		*r.u. per 100 cc.*	*r.u. per 100 cc.*	*r.u. in 24°*	*r.u. in 24°*						*r.u. in 24°*	*r.u. in 24°*
32	3/23/32	6/14/32	6 mos. Toxaemia	166/108	L.T.	34.0	6.1	Age 27 para I. Oedema and dizziness. Follow up: 2/24/33. B.P. 132. Alb. 0	200	400	1,500	2,500		—	—		—	600	800
33	1/14/33	4/10/33	6 mos. Toxaemia	125/80	L.T.	25.0	2.5	Age 26 para I. Oedema. 1/14–27/33: Routine treatment—lost 15 lbs.	66.6	100	1,730	2,300				—		2,800	4,250
	1/27/33	4/10/33	6½ mos. Toxaemia	105/65	T	23.0	3.5	Forced delivery 2/24/33	125	200	2,800	3,200				+		1,160	1,660
34	4/28/33	7/13/33	6½ mos. Normal	102/68	0			Age 19 para I	66.6	100	900	1,060		—	++±		+++	2,700	3,500
35	5/12/33	8/10/33	6 mos. Diabetes insipidus	120/70	0			Age 28 para I. No oedema. Has been taking pituitrin regularly—3 pledgets intranasally daily	66.6	100	405	540			++±	+++		1,800	2,700
36	6/ 9/33	9/10/33 9/11/33	6 mos. Normal	110/	0			Age 30 para II. First pregnancy normal			800 820	1,060 1,100						3,500 3,900	4,300 4,900

symptoms and served as controls. Case 32 had high prolan and low oestrin in both serum and urine. Case 33 was excreting an excessive amount of prolan when the first analyses were performed and had an abnormally low level of oestrin in the serum. Her excretion of oestrin, however, was normal, as was also the serum prolan. Two weeks later the prolan of both serum and urine had increased and the oestrin excretion had decreased. In normal pregnancy a change in the opposite direction would be expected.

Case 35, in spite of diabetes insipidus, is well and apparently passing through an uneventful pregnancy. During the first 5 months larger quantities of pituitrin (intranasally) were necessary than before conception, but since then less has been needed. The serum prolan and oestrin are within normal range but their excretion is somewhat low. This case is particularly interesting in the light of the finding by Anselmino and Hoffmann (11) of a posterior pituitary-like substance in the blood of toxaemic patients. If their hypothesis is correct, that an excessive pressor and antidiuretic principle from the posterior lobe plays a dominating rôle in toxaemia, one would expect that a patient receiving pituitrin continuously throughout pregnancy might develop toxic symptoms, unless, as is likely in this instance, the drug is merely replacement therapy.

Case 37, table 4, was a woman whose urine and serum were analyzed monthly from early in pregnancy until term, and who developed oedema, albuminuria and an elevation of blood pressure during the last month before delivery. We have made monthly analyses on a normal woman throughout gestation and found, as has been reported by others (12, 13), a continuous increase in oestrin from the beginning of pregnancy to term and a continuous decrease in prolan from the fourth month on. From the second through the fifth month, case 37 revealed nothing abnormal; i.e., there was a continuous increase in oestrin and a decrease in prolan beginning after the third month. At six and one-half months, however, although the patient was well, there was a marked increase in the serum prolan and a decrease in urinary oestrin, the situation found to be typical of a toxaemic condition. It is interesting that this woman did not develop toxic symptoms until a month and a half after this abnormal increase in prolan began.

Table 5 presents the results on 5 patients who had eclampsia. From only one of these (case 40) was it possible to obtain 24-hour specimens of urine. Two such specimens collected by an in-lying catheter were only 240 and 275 cc. respectively and the creatinine was only about two-thirds of the calculated quantity. It is probable, therefore, that kidney retention accounts for the low hormone excretion. In all 5 cases the serum prolan was considerably above the limits of normal and the average figure is higher than the average for the cases of toxaemia without convulsions. Some of

TABLE 4

Monthly analyses throughout pregnancy of specimens from a patient who developed toxic symptoms during the last month

Case 37. Last cta. 10/26/32. Due 8/2/33

DATE OF SPECIMEN	PERIOD OF GESTATION	BLOOD PRESSURE	URINARY ALBUMEN	COMMENTS	ASCHEIM-ZONDEK TESTS				ALLEN-DOISY TESTS							
					Serum		Urine		Serum						Urine	
					Positive	Negative	Positive	Negative	1 cc.	2 cc.	3 cc.	4 cc.	5 cc.	6 cc.	Positive	Negative
1/12/33	2½ mos.	130	0	Age 33 para II. Severe toxaemia at 8th month of last pregnancy. Some vomiting this time	333	500	1,400	2,100					−	±	700	1,000
2/13/33	3½ mos.	118	0	Vomiting but not severe	333	500	2,100	2,750					++	+++	760	920
3/16/33	4½ mos.	120	0	Still vomiting	125	200	900	1,200			++	+++	+++		2,700	3,300
4/11/33	5½ mos.	112	0	Well	100	125	640	800		+	+++				2,100	2,700
5/ 9/33	6½ mos.	128	0	Well	500	1,000	640	750		±	++±				1,070	1,500
6/ 5/33	7½ mos.	118	0	Well	200	333	730	960	−	++	+++				2,400	3,200
6/27/33	8 mos.	126	S.V.T.	Well. On 7/3/33, however, began showing S.T. alb. and oedema of ankles	500	1,000	3,800	5,300	++	+++					5,900	7,400
7/10/33	8½ mos.	138	S.P.T.	Oedema. Delivered 7/22/33. Had B.P. around 140 for last 2 weeks and S.P.T. alb.			5,000	7,500							3,300	5,000

TABLE 5

Eclamptics

CASE	DATE OF SPECIMEN	DATE WHEN DUE	HOSPITAL DIAGNOSIS	BLOOD PRESSURE	URINARY ALBUMEN	BLOOD CHEMISTRY		COMMENTS	ASCHEIM-ZONDEK TESTS				ALLEN-DOISY TESTS						
									Serum		Urine		Serum					Urine	
						N.P.N.	Uric acid		Positive	Negative	Positive	Negative	1 cc.	2 cc.	3 cc	4 cc.	5 cc.	Positive	Negative
						mgm. per 100 cc.	*mgm. per 100 cc.*		*r.u. per 100 cc.*	*r.u. per 100 cc.*	*r.u. in 24°*	*r.u. in 24°*						*r.u. in 24°*	*r.u. in 24°*
38	12/18/32	1/ /33	8½ mos. Eclampsia	170/80	H.T.	29.0	4.5	Age 27 para I. Convulsions. Delivered of twins by Caesarian 12/18/32	333	500			±	++±	+++				
39	1/16/33	1/17/33	9 mos. Eclampsia	140/90	T			Age 20 para I. Convulsions. Convulsion just before blood taken	500	1,000			−	−	++				
40	5/ 3/33	5/ 3/33	8½ mos. Eclampsia	150/100	H.T.	32.0	4.5	Age 19 para I. Two convulsions. First blood taken before any R. Then put on restricted fluid with sod. amytal	1,000	2,000	500	800		+	+++			200	400
	In labor 5/ 5/33	Delivered 5/ 5/33		118/80				Second blood taken while in labor	1,000	2,000				−	±		±±	950	1,360
41	5/17/33	Delivered 5/20/33	8 mos. Eclampsia	155/90	L.T.	35.0	5.3	Age 36 para I. One convulsion on 5/17/33. R—Morphine, $MgSO_4$, fluids and forced delivery	200	333				±	++±				
	5/19/33								200	333				−	−	−			

42	6/12/33	6/26/33 Delivered 6/13/33	8½ mos. Eclampsia	180/120	H.T.	27.0	3.8	Age 22 para II. First pregnancy normal. Blood taken before R—not in labor Three convulsions—forced delivery	125	200			++±	+++±	+++±	++			
Average.....									480	900									

the cases without convulsions, however, had as much prolan in their serum as some of the eclamptics. It is apparent that the degree of excess of prolan does not always run parallel with the severity of symptoms. The serum oestrin of 2 of the eclamptics (cases 38 and 42) was normal. Blood was collected from cases 40 and 41 on 2 different occasions and although the prolan had not changed in 3 days the oestrin had decreased. Margaret Smith (12) found that normally the blood oestrin increased markedly shortly before and during labor. The unexpected decrease of oestrin in these 2 patients, therefore, indicates an abnormal situation.

Discussion. The question of the source of the excessive gonad-stimulating hormone in toxaemia immediately presents itself. Is it hypophyseal or placental? We have injected fresh extracts of the serum both from these patients and from normal women in late pregnancy into hypophysectomized rats[2] whose ovaries were fully atrophied. In all cases extracts of the amount of serum equivalent to 4 rat units of prolan have been employed. So far we have been unable to demonstrate any quantitative or qualitative gonad-stimulating difference between these serums, since in every instance there has been enlargement of the ovaries and hypertrophy of interstitial cells, and occasionally development of new, though small, corpora lutea. If the excessive prolan of toxaemic serum were hypophyseal one would expect a more marked effect upon the ovaries of hypophysectomized rats. These results are not conclusive but suggest that the hypophysis does not elaborate the excessive prolan in toxaemia and that therefore the placenta may. They also indicate that part of the prolan in normal late pregnancy is hypophyseal. Philipp (15) has contended that normally prolan is elaborated by the placenta rather than the hypophysis, since placental transplants are active in producing changes in the ovaries of immature mice, whereas transplants of the hypophyses of pregnant women are inactive. Anterior lobe transplants from normal women are active. Evans et al. (18) have offered evidence that urinary prolan, presumably from the placenta, "activates" an hypophyseal factor. Zondek (16), on the other hand, has advanced convincing arguments in favor of the hypophysis as the source of prolan in pregnancy with the placenta as merely a place of storage. In a recent series of papers (24, 25, 26) Smith and Leonard report that antuitrin S, an extract of pregnancy urine, has definite effects upon the gonads of hypophysectomized rats. Further, Hill and Parkes (17) have produced ovulation in hypophysectomized rabbits with untreated urine of pregnancy. It seems to us likely that Reichert et al. (19) obtained negative results in hypophysectomized dogs and rats because they employed a refined extract in which the labile hypo-

[2] We are much indebted to Dr. K. W. Thompson, Cushing Fellow in Surgery at the Peter Bent Brigham Hospital, who, because of greater experience, has hypophysectomized our rats.

physeal factor may have been destroyed. The work of various investigators (18, 20, 21, 27) has indicated that the gonadotropic hormone extracted from the urine of pregnancy differs in its physiological actions from that of anterior lobe extracts. As stated by Smith and Leonard (24, 25, 26, 27), the relationship between prolan and the hypophyseal gonadotropic factor seems to become more rather than less obscure, but we are led to believe that *both* a placental and hypophyseal factor may be present in the blood and urine during pregnancy. If this is so, many of the apparently contradictory findings would be explained.

Since the hypophysis has been found necessary for the survival of pregnancy in animals (16), presumably through its effect on their ovaries, which are also needed to maintain pregnancy, and since in humans the ovaries are not essential after the placenta is once established (22, 23), we wonder if in humans there may not be a *direct* action of the hypophysis upon the placenta. This conception, together with the fact that placental hormones are known to affect the hypophysis, establishes so close a relationship between these two organs that any abnormality in one would presumably influence the functional activity of the other.

The conception of a placental endocrine abnormality is appealing because of the fact that the placenta may not be disregarded as the possible source of factors causing toxaemia, since improvement follows its delivery. The low oestrin found in the majority of the above toxaemics is indeed a placental deficiency, since that organ is admittedly the source of practically all oestrin in human pregnancy (22, 23). The administration of oestrin to case 21 was apparently effective in depressing the excessive prolan. This result may be interpreted either as inhibition of hypophyseal gonadotropic activity or as direct inhibition of placental elaboration of prolan.

The pituitrin-like substance which Anselmino and Hoffmann (11) have isolated from toxaemic patients points towards pituitary dysfunction. In attempting to produce toxic symptoms in rabbits we have injected large amounts of prolan (Parke, Davis & Company "Antuitrin S") and an anterior hypophyseal extract (Parke, Davis & Company #094512-C) which was potent in causing ovarian changes and lactation. The animals that had antuitrin S either miscarried or had death of at least part of the pregnancy in utero with prevention of normal delivery at term. The hypophyseal extract always caused death of pregnancy. We were therefore forced to use non-pregnant animals and have been unsuccessful in producing toxic symptoms. One interesting, though coincidental, observation, however, was that the hypophyseal preparation brought about a marked decrease in urinary output. It did not cause a rise in blood sugar, thus eliminating the possibility of its containing pituitrin. Injections of antuitrin S and urine of normal pregnancy did not have any antidiuretic effect, but in 2 instances a decrease in urine followed injections of follutein

(Squibb). A decrease in urinary excretion was also noted in a spayed rabbit that received daily 20 cc. of the plasma from a patient with eclampsia upon whom a plasmapheresis had been performed. In this instance, moreover, there was no rise in blood sugar. This single experiment is in agreement with the finding of Anselmino and Hoffmann that the blood of toxaemic patients contains an antidiuretic substance. Our obtaining an antidiuretic effect with anterior lobe extract, however, and the failure of both anterior lobe extract and toxaemic plasma to cause a rise in blood

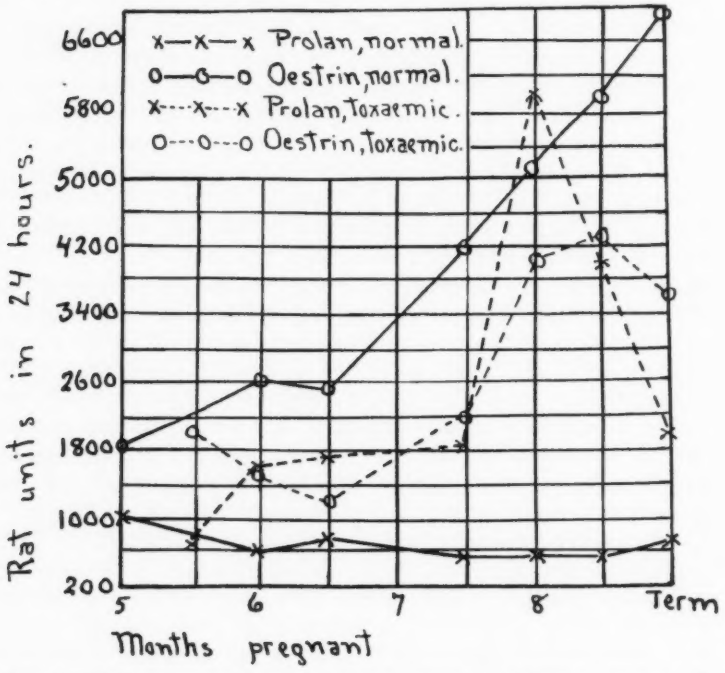

Chart 1. Urinary excretion of prolan and oestrin during late pregnancy. Composite curves of 24-hour urines of 15 normal and 21 toxaemic women.

sugar cast doubt on their conclusion that they were dealing with a pituitrin-like principle.

Perhaps the most convincing evidence for involvement of the pituitary in eclampsia has been brought forth recently by Cushing (28), who reports that in the few fatal cases of eclampsia and hypertension that he has had an opportunity to study he has found a heavy invasion of the posterior lobe of the pituitary by basophilic elements from the pars intermedia. The indications are that the basophilic cells are responsible for the production

of the hypophyseal gonadotropic hormone. However, we have so far been unable to get any evidence from experiments on hypophysectomized rats that the excess of gonadotropic hormone in toxaemia is hypophyseal rather than placental. The close interrelationship between the hypophysis and placenta, nevertheless, makes it seem likely that, whatever the source of excessive prolan, our findings are in some way closely connected with those of Anselmino and Hoffmann and of Cushing. Whichever organ is primarily at fault, it seems safe to conclude that the high prolan and low oestrin in toxaemic patients, the pituitrin-like substance in the blood and the pathological findings in the pituitary are all manifestations of a disturbed hypophyseal-placental relationship which may very well be associated with the etiology of this condition. Further, whether the endocrine changes described in this paper are causal or coincidental, the findings are sufficiently consistent to make it safe to conclude that a quantitative imbalance between prolan and oestrin is characteristic of the toxaemias of late pregnancy (see chart 1).

SUMMARY

From 42 women in the last third of pregnancy 46 sera and 44 24-hour specimens of urine have been analyzed for their content of the gonadotropic hormone (prolan) and oestrin. Of these women, 15 were normal, 22 had toxic symptoms without convulsions and 5 had eclampsia.

One of the patients with toxic symptoms was definitely nephritic and showed normal levels of prolan and oestrin. In no case diagnosed as toxaemia or eclampsia were both oestrin and prolan within the limits of values found in the normal women at the same period of gestation. In a number of the toxaemic women, however, there was unquestionably a nephritic element. All but one of 26 toxaemic and eclamptic patients, or 96 per cent, showed excessive amounts of prolan in the urine and serum, and 18 of them, or 69 per cent, had subnormal levels of oestrin. The urines and sera of one toxaemic patient were analyzed monthly from the second month to term. During the sixth month an abnormal increase in prolan and decrease in oestrin appeared, which continued with fluctuations until delivery. This patient developed no toxic symptoms until the eighth month.

The analyses of specimens from the patients who were more than a month and a half from term and from the nephritic case are not included in the following average figures on the other 35, who were all a month and a half or less from term.

From these results it is concluded that *a quantitative imbalance of these two hormones due to excessive amounts of prolan and, less consistently, to subnormal levels of oestrin is typical of the toxaemias of late pregnancy.*

Average figures

HOSPITAL DIAGNOSIS	NUMBER OF CASES	SERUM PROLAN	URINE PROLAN	SERUM OESTRIN, AMOUNT REQUIRED TO GIVE POSITIVE TEST	URINE OESTRIN
		r.u. per 100 cc.	*r.u. per 24[b]*		*r.u. per 24[c]*
Normal	12	50	560	3 cc. or less in 12 cases	5,800
Toxaemia	18	250	3,600	4 cc. or more in 12 cases 3 cc. or less in 6 cases	3,700
Eclampsia	5	480		4 cc. or more in 3 cases 3 cc. or less in 2 cases	

The question of the source of the excessive prolan and the possibility of an hypophyseal-placental disturbance in the toxaemias of late pregnancy are discussed.

The patient with diabetes insipidus (case 35, table 3) required less pituitrin during the middle of gestation, but as pregnancy advanced greatly increased doses were necessary, particularly during the last few weeks. She delivered normally and was able to nurse. The second week after parturition she was able to reduce the dosage of pituitrin, but not to her usual level.

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ZINC IN THE NUTRITION OF THE RAT[1]

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It is only natural that zinc which is so universally distributed in biological materials (1, 2, 3, 4) should be included in studies of the rôle of minor inorganic elements in nutrition. An insight into the biological significance of any inorganic element can only be obtained when animals are fed rations which contain very little or none of the element in question. This type of experiment was first attempted in studies on zinc by Bertrand and Benzon in 1922 (5). They fed mice a purified ration which was thought to be free of zinc. The animals lived for an average of only about 20 days because no attempt was made to supply the vitamins which had been destroyed during the purification of the ration. A similar group of animals fed the same ration to which a small quantity of zinc sulfate had been added lived 25 to 50 per cent longer.

McHargue (6) in 1926 carried out a similar experiment with rats. His ration was not purified but was chosen from materials which he felt would yield a zinc low ration. Copper, zinc and iron were added to this ration but again the vitamin supply was deficient. Slightly better growth was made by the animals on the diet which had been fortified with the metals. These two experiments offer only a slight indication that zinc is in any way connected with the growth of experimental animals.

A more complete study was made by Hubbell and Mendel in 1927 (7). They fed mice a diet which supplied only 0.005 mgm. of zinc per animal daily. Special attention was given to the supply of the known vitamins. The growth on this diet was compared with that obtained on the same diet plus small quantities of zinc, and with that obtained on an unpurified diet which allowed a zinc intake per animal of 0.318 mgm. daily. The growth differences between the various groups were very small. Slightly better growth was noted in the group ingesting 0.02 gram of zinc per 100 grams of ration than in the group receiving 0.04 gram per 100 grams of ration. The fact that the growth differences among the various groups were no larger than those often experienced in vitamin deficiency experiments made

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it difficult for them to draw definite conclusions in regard to the beneficial effect of zinc on growth. Nevertheless, they state that, "It is not unlikely, however, that there is some variation in growth with varying amounts of zinc and that the metal is not merely an accidental factor in the nutrition of the mouse."

Thus it is seen that the indications in the literature of the beneficial effect of zinc on the growth of rats and mice are very meager. The purpose of this research was a further attempt to determine whether or not zinc does exert a beneficial rôle in the nutrition of the rat.

EXPERIMENTAL. A complete ration for the rat should contain an ample supply of carbohydrates, proteins, fats or fatty acids, vitamins, minerals, and water. The protein must be of such quality as to contain the essential amino acids. The fat requirement is apparently rather small and it is questionable whether the rat needs more than one or two of the fatty acids in its food. The water soluble vitamin B complex must be supplied and of the fat soluble vitamins only A and D are required. Vitamin E may not be a requisite for short time growth experiments. In a highly purified diet the salt mixture should contain all of the known essential mineral elements and it is advisable to add the calcium and phosphorus in about the ratio of 2:1.

In purifying the various ingredients of the ration large quantities of water were necessary. The distilled water from the tap was found to be not sufficiently free from metals for this purpose and consequently all of the water used as well as some of the other reagents were redistilled from glass.

Carbohydrate. A market grade of corn starch yielded to purification with respect to zinc by the following procedure: Three pounds of the starch were suspended in 12 liters of 0.05 per cent HCl (reagent quality) in a large glass battery jar. After the starch settled to the bottom the supernatant liquid was drawn off by suction and the starch again suspended in 12 liters of the dilute acid. This procedure was repeated 4 times, but the last washing was carried out with redistilled water in order to dispose of most of the acid before drying. After filtering the starch on a large Buchner funnel it was washed 2 or 3 times with redistilled alcohol and dried in large evaporating dishes in a drying room (temperature 40°).

A 200 gram sample of starch so purified contained less than 0.05 mgm. of zinc as none could be detected by the Todd and Elvehjem method for the determination of zinc (8), which was used throughout in this work.

Protein. Inasmuch as casein is easily prepared and purified from skim milk in glass containers this source of protein was utilized in the ration. The procedure developed for its preparation was as follows:

One and one-half gallon of skim milk was placed in a 14 liter glass battery jar and the jar was almost filled with redistilled water. One per cent HCl

was added slowly with vigorous stirring until the casein was completely precipitated in a fine granular form. After settling, the supernatant liquid was drawn off with suction and about 8 liters of redistilled water were added to the casein. After stirring and allowing the casein to settle, this supernatant liquid was drawn off. The jar was again filled with redistilled water and enough redistilled ammonia added to dissolve the casein.

Hydrochloric acid was again added to precipitate the casein, and after the supernatant liquid was removed the casein was again washed with redistilled water. This procedure of dissolving, precipitating and washing was repeated four times in all and then the product was filtered on a large Buchner funnel and partially dried by repeated washings with redistilled alcohol, after which the material was placed in a drying room in large evaporating dishes until completely dried. Such a product was by no means free of zinc, in fact it was found that 7 reprecipitations under the conditions described yielded a product of no lower zinc content than one treated 4 times. Analyses of several samples showed variations of from 2.7 to 5.0 mgm. of zinc per kilo on the dry basis.

Egg white, to the extent of 5 per cent was also used as a source of protein and its preparation is described under vitamin B_2.

Fat and vitamins A and D. The fat was supplied in the ration in the form of Wesson oil in which was dissolved sufficient Haliver oil to supply the fat soluble vitamins. The Haliver oil contained 30,000 units of vitamin A (U. S. P. X.) and 3,333 units of vitamin D (Steenbock) per gram. When 2 cc. of this oil were dissolved in 48 cc. of Wesson oil, one drop of the mixture (60 drops per cc.) contained approximately 20 units of vitamin A and 2.2 units of vitamin D. This amount was fed daily to each rat with a medicine dropper.

In the early part of the first experiment, the Haliver oil was dissolved in redistilled ether and dried over the ration in such quantity that 10 grams of ration contained amounts equivalent to these values. This procedure led to the destruction of a large portion of the vitamin A (through oxidation by the iron in the ration) and was consequently abandoned.

Vitamin B_2. Carefully handled egg white which had previously been hard boiled was dried before a fan in large evaporating dishes and used to the extent of 5 per cent of the ration as a source of vitamin B_2. That it is a good source of the antipellagric factor was shown by Chick, Copping and Roscoe (9). The zinc content of the dried substance was found to be very low.

Vitamin B_1. Considerable difficulty was encountered in the production of a source of this factor which was potent and low in zinc. Failure resulted in a number of attempts to purify extracts of yeast and rice polishings with respect to zinc. The following procedure, however, proved fairly

successful and provided a potent source of the antineuritic vitamin with a minimal zinc content.

Three kilos of rice polishings were suspended in 12 liters of 25 per cent alcohol and allowed to stand for 3 days with occasional stirring. The volume of the supernatant liquid was reduced in vacuo to one liter at a temperature not exceeding 60°C. The bulk of the protein was removed at this point with lead acetate, and the lead was taken out with H_2S. This concentrate was then reduced in vacuo to 200 cc. The resulting brown syrupy liquid had a pleasing odor and taste and contained sufficient vitamin B_1 to supply the requirements of this factor for growing rats when fed at a level of 3 to 5 drops daily (40 drops per cc.).

As a means of assaying the preparations, young stock males 25 days of age were placed on a vitamin B_1 deficient diet until growth had ceased which required about 3 weeks. At this time the vitamin preparations were administered to the extent of 3 drops daily per animal. Two preparations were assayed and have been sufficient to supply this factor throughout the experimentation. Gains in weight of from 40 to 50 grams in 4 weeks were made by each animal receiving 3 drops daily of either of the vitamin preparations. The negative controls continued to lose weight and died after 5 to 6 weeks on the restricted diet.

The zinc content of these preparations was found to be very low, 1 cc. containing only 8 gamma. Thus the feeding of a few drops of these preparations daily would add an insignificant amount to the total zinc ingestion.

Mineral mixture. The following simplified salt mixture was used in all the experiments:

Salts 45T

$MgSO_4$	8	per cent
NaCl	20	per cent
KCl	10	per cent
Calcium phosphate	60	per cent
Salts 42T	2	per cent

Salts 42T

$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$	89.8	per cent
$CuSO_4 \cdot 5H_2O$	5	per cent
$MnSO_4 \cdot 4H_2O$	5	per cent
KI	0.2	per cent

The magnesium, sodium and potassium salts were Kahlbaum products of a high order of purity. No purification of these was necessary. The compounds in salts 42T were not purified inasmuch as they were present in the ration to such a small extent.

A large number of calcium salts were found to be contaminated to a

considerable degree with zinc, and the purest samples of dicalcium phosphate would not yield to purification by solution in pure HCl and precipitation with redistilled ammonia. The following procedure yielded a mixture of calcium phosphates which showed no zinc in a 10 gram sample by the method of analysis employed.

Clear crystals of calcite ($CaCO_3$) were washed in dilute HCl and finely ground in a glass mortar. The powdered carbonate was suspended in redistilled water in a beaker and warmed. Small portions of reagent quality phosphoric acid were added from time to time until a slight excess of the calculated amount had been introduced. When the reaction was completed the precipitated calcium phosphate was filtered and washed free of acid with redistilled water. The salt was next dissolved in concentrated HCl and the solution diluted. Upon the addition of purified ammonia, calcium phosphate was reprecipitated. After washing the salt several times with redistilled water it was partially dried with redistilled alcohol and then completely desiccated in a drying room.

The complete zinc low ration contained the following ingredients:

Ration 9—Low zinc

Casein	14 per cent
Egg white	5 per cent
Starch	77 per cent
Salt mixture 45T	4 per cent
Redistilled water	ad libitum
Vitamin supplements daily	

This ration contained 0.56 per cent of calcium and 0.32 per cent of phosphorus.

The control ration was made by adding ZnO to this diet.

Ration 9A—With added zinc
Ration 9 plus 5.0 mgm. of zinc
as ZnO per 100 grams of ration.

In preparing the ration each ingredient was finely ground in a glass mortar and thoroughly mixed in a large evaporating dish. Two kilos were usually prepared at one time, one-half having zinc incorporated into it by mixing the ZnO with a small portion of the ration and incorporating this into the remainder.

Analyses of 100 gram samples of the zinc low ration showed a zinc content of 1.6 mgm. per kilo which would be equivalent to 16 gamma of zinc per 10 grams of ration, the average daily consumption of a growing rat. In the experiments in which 2 cc. of milk were fed daily with the dry ration, the zinc ingestion of a rat eating 10 grams of feed daily would be approximately 24 gamma per day.

10 grams ration 9	16 gamma
Vitamins A, D and B_1	2 gamma
2 cc. milk[2]	6 gamma
Total	24 gamma

Only one variation of this ration was used. In an attempt to increase the vitamin B_2 content, 0.5 per cent of Lilly Liver Extract 343 was added and the ZnO replaced by $ZnCl_2$ in the control diet in hopes that this salt might be more available to the animals.

The composition of this ration was as follows:

Ration 12—Low zinc

Casein	14 per cent
Egg white	5 per cent
Starch	76.5 per cent
Salt mixture 45T	4 per cent
Lilly liver extract 343	0.5 per cent
Redistilled water	ad libitum

Vitamin supplements daily.

The control ration for this second zinc low diet was prepared by dissolving $ZnCl_2$ in purified ether and drying the solution over the ration. The amount of the metal incorporated was the same as in ration 9A, i.e., 5.0 mgm. per 100 grams of ration. The liver extract showed on analysis a zinc content of 40.0 mgm. per kilo. This added liver supplied 20 gamma of zinc per 10 grams of ration or more than an equivalent amount of ration without the liver extract.

Cages and feed containers. The cages used in this work were made of monel metal screening on a wooden frame, so constructed that the wood was entirely covered with the screening in order to lessen the possibility of gnawing by the rats. The arrangement was such that each cage could be divided into three compartments by movable partitions. Each compartment was about 10 inches square and 12 inches high. The bottoms were made of 3 mesh screening and no trouble from coprophagy was experienced.

Glazed porcelain dishes 3 inches in diameter were used as feed containers. These were covered with monel metal screening which was cut away in the center. The dishes were placed in one pound glazed porcelain jars to lessen the loss of feed from scattering by the rats. The milk and redistilled water were also administered in small porcelain dishes.

With this type of diet the number of animals which may be kept under

[2] The milk used in this work was carefully handled and came directly from the dairy barn to the laboratory each morning in a porcelain can. A series of analyses on samples of this milk gave results of from 2.68 to 2.76 mgm. of zinc per liter. This is equivalent to less than 3 gamma per cubic centimeter.

experimental observation at one time is necessarily limited. The cages were expensive and the preparation of the ration was not only laborious but required approximately 100 liters of redistilled water to produce 1000 grams.

The animals in each of the 6 experiments were from the same litter. The young with their mother were placed in one of the small compartments of the monel cages at 10 to 12 days of age and kept there until the beginning of the experiment in order to lessen the ingestion and storage of zinc from the stock colony cages which are made of galvanized iron screening. The mother was fed water, milk and the stock colony ration in high glazed porcelain dishes. When the rats were about 17 days old the zinc low ration was substituted for the stock colony ration. At the age of 23 to 27 days the litter was weaned and divided into two groups, one being fed the zinc-low ration and the other the same ration containing added zinc. These will be referred to hereafter as group A and group B respectively.

The dishes used to feed milk and water were washed daily and rinsed with distilled water. The dry ration and water were kept before the animals at all times. Weekly weight records were kept of all the rats.

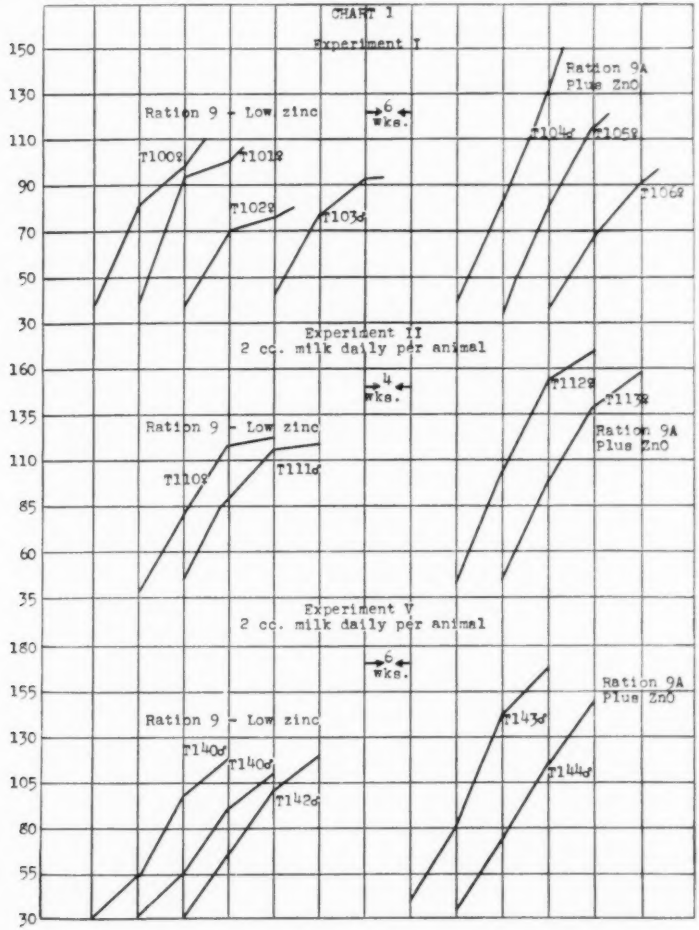
In all but the first experiment each rat was given 2 cc. of milk daily. Rations 9 and 9A were fed in experiments I, II, V, and VI, and rations 12 and 12A in experiments III and IV. After the first 8 weeks of the first experiment, at which time ophthalmia appeared, vitamins A and D were administered daily rather than as a part of the ration. The latter method was also used in all of the subsequent experimentation.

Results. In order to conserve space the growth curves for only 3 of the 6 experiments are reproduced. In chart 1 the growth curves are found for experiments I, II, and V. In the first experiment growth was slow due in part to the vitamin A deficiency and in part to the fact that the animals received no milk. During the first six weeks the differences in growth between the animals on zinc low and zinc fortified rations were small but after that time the latter animals showed considerably better growth.

In experiment II growth was much more rapid due undoubtedly to the presence of milk in the diet. With this rapid growth it is logical to expect any manifestations of a zinc deficiency to appear earlier. The large differences in growth between group A and group B uphold this assumption. Experiments V and VI were exact duplicates of this experiment and the growth curves are similarly comparable. Of these only no. V is reproduced. Growth in this group was retarded during the first few weeks from an insufficient supply of vitamin B_1. When the daily dose was increased from 3 to 5 drops per animal growth was resumed.

Liver extract was added to the rations used in the third and fourth experiments which greatly increased the zinc ingestion. It is possible that the amount of zinc allowed under this regimen was approaching the mini-

mum requirement, for after 10 weeks the weights of the animals of both groups in these two experiments were quite comparable. However, if the weights of the rats are compared, males with males and females with females, in the early part of the experiment, the rate of growth was far



greater in group B, the zinc fortified ration. At 5 weeks the male of group B weighed 140 grams while the two males of group A had reached weights of only 100 and 107 grams respectively. Experiment IV showed weight differences of the same magnitude, 92 grams and 50 grams at 4 weeks for a female of group B (with zinc) and a female of group A (low zinc) respectively.

A beneficial effect on the growth of rats upon the addition of small amounts of zinc salts to the zinc low ration has been noted in each of the six experiments. It is strongly inferred that this metal is in some way concerned with the growth processes.

The only pathological condition noted in the animals on zinc low rations was a loss of hair. There are a number of interesting reports in the literature (10–15) regarding similar phenomena in rats and other animals on incomplete rations. There has been no suggestion as far as we know that zinc is functional in the development of a normal fur coat.

In each experiment this abnormality has appeared, in some cases to a severe and in some cases to a less severe extent. It is characterized by a loss of hair first around the head and shoulders. In the less severe cases it was more or less localized about these areas and including the under portion of the neck and chest. Figure 1 shows a female of group A, experiment I,

Fig. 1. Female of group I, experiment I, after 17 weeks on experimental diet. Entire ventral side of body is bare.

after 17 weeks on the restricted diet. This is considered as a severe case inasmuch as the loss of hair spread caudally until the entire ventral side of the body became bare.

Occasionally small bare patches were noted on the backs or sides of the animals, but these areas usually developed new hair. Where the hair persisted it was shaggy and thin.

The time required for the development of this phenomenon varied among the different experiments and to a lesser extent among the individuals of the same experiment. In general it may be said that the more rapid the growth of the animal the sooner the loss of hair developed. In experiment II bare spots appeared after two weeks on the purified rations while in the first experiment no loss of hair was noticed for eight weeks.

In no instance was there noted a thinning or loss of hair in the control animals, their pelts appeared normal in every respect.

After 15 weeks on the experimental diet two of the animals from group A, experiment I, were placed on the control diet (ration 9A). One of these animals was completely bare on the ventral side of the head, shoulders and chest. There were also bare patches on the inner sides of the back

legs. Considerable improvement in this condition was noted after 5 weeks, although the animal weights remained rather constant. After 7 weeks the bare spots were almost completely covered.

DISCUSSION. From the low maximum weights attained by all of the animals (172 gr. highest weight recorded) it seems evident that the ration was not entirely complete even though all of the known essential nutritional factors were supplied. That an additional factor or factors do exist in the water soluble B complex is rather definite at present although their nature has not as yet been elucidated. Barnes, O'Brien and Reader (16) have described a vitamin B_4 deficiency in their rats. One of the symptoms noted was the tendency for the animals to draw their four paws together when resting with the resulting appearance of a hunched back. Such a position has been noted in some of the animals which have been on a subminimal zinc level for long periods. It would be inadvisable at present to attempt to correlate these symptoms with those reported by Reader.

Another possibility which may have accounted for the low maximum weights was a lack of further inorganic elements such as aluminum, boron, arsenic, and others. No analyses for the minor inorganic elements with the exception of zinc were made on the rations. But it is felt in view of the extreme precautions employed in the purification of the ration, that the content of all inorganic elements not added may have been exceedingly low.

The vitamin preparations, including A, D and B_1 were very palatable and were consumed greedily when administered. It is certain that no loss occurred through repulsion. It is also certain that the quantity received by each animal daily was exactly the same.

The quantity of vitamin B_2 only was dependent on the amount of ration consumed. That the symptoms exhibited by the rats on the zinc low rations were not manifestations of a deficiency of this entity through lowered food consumption seems clear. One of the outstanding symptoms of a vitamin B_2 deficiency in a rat is the occurrence of a scaly condition about the feet and legs. This was not observed in any of the experimental animals.

In experiments now in progress in which food consumption records are kept, the data indicate that the differences in food consumption on the zinc-low and zinc fortified rations are not great. Consequently, it would not be plausible to explain the growth differences on the basis of a lowered vitamin B_2 intake.

During the preparation of this work for publication a report by Newell and McCollum (17) appeared on the necessity of zinc in the diet of the rat. These authors also used a purified synthetic ration which they claimed to contain 10^{-7} part of zinc. No differences in growth were noted in animals on this ration and animals eating the same ration to which small amounts

of zinc had been added. Their results are not in agreement with those reported in this paper. At present we have no adequate explanation for these divergent data.

SUMMARY. Young rats at the weaning age were placed in monel metal cages and fed a highly purified synthetic ration which contained only about 1.6 mgm. of zinc per kilo of dry weight. All of the known vitamins and essential mineral elements were supplied. Small quantities of milk (2 cc. daily per animal) were used as a supplement to this ration, which though apparently not entirely complete, allowed sustained but subnormal growth when ZnO or $ZnCl_2$ was added to the extent of 5.0 mgm. of zinc per 100 grams of ration. When this ration was fed without the added zinc the growth of the rats was markedly inferior both as to rate of growth and maximum weight obtained.

Subminimal levels of zinc in the ration invariably led to a loss of hair in the rats about the neck and shoulders, which in the extreme cases resulted in the complete baring of the entire ventral side of the body. What appeared to be normal fur development was exhibited by the animals fed the ration to which zinc had been added.

CONCLUSIONS

1. On a synthetic diet low in zinc the rate of growth of rats was accelerated by the addition of salts of this metal.

2. On the synthetic diet low in zinc there was interference with the development of a normal fur coat in the rat.

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FURTHER OBSERVATIONS ON THE VITAMIN A CONTENT OF THE LIVERS OF DEPANCREATIZED DOGS AND ITS RELATIONSHIP TO THE SYMPTOMS OCCURRING IN THESE ANIMALS[1]

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In a previous paper (1) we described a group of symptoms occurring in depancreatized dogs, which we thought suggestive of vitamin A deficiency. The livers of these animals contained relatively small amounts of the vitamin. Normal dogs fed a diet deficient in vitamin A (1, 2, 3) developed similar symptoms. It is true, however, that certain of the symptoms, e.g.: loss of hair, scaliness of the skin, and sores, are found in other conditions. Rats for example, on a diet deficient in B_2 (G) develop loss of hair about the eyes and across the shoulders (4). Burr and Burr (5) and others (6) have reported scaliness of the skin in rats on diets deficient in the essential fatty acids. Whipple (7) reports a group of symptoms in dogs fed a diet containing oxidized fat, which are similar in certain respects to those we observed in our depancreatized and A deficient dogs.

In order to rule out some of the conflicting causes of the syndrome observed in depancreatized dogs we have studied the effects of an adequate amount of B_2, of cod liver oil, of lecithin and cod liver oil and of lecithin alone, added to the diet. Lecithin was given because of the observations of Hershey and Soskin (8) and Best and Hershey (9). They felt that in depancreatized dogs there is a disturbance of phospholipid metabolism which is corrected by adding lecithin to the diet. Some observers have suggested a relationship between the metabolism of vitamin A and the phospholipids. Although no satisfactory proof of this has been presented, we thought it advisable to feed lecithin alone and with cod liver oil. One dog was given cystine. This was to check a statement of Hedon's (10) that cystine and sulphur, the amounts not mentioned, prevented loss of hair in one of his depancreatized dogs. We also gave cystine and an excess of B_2 to normal dogs on an A deficient diet. These results will be reported later

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[2] Henry Strong Denison Fellow-in-Medicine.

TABLE 1

Summary of diets, observations and findings

DOG NO.	DIET AMOUNTS IN GRAMS ALL DOGS RECEIVED BONE ASH 4 GRAMS AND SALT MIX 3 GRAMS	ADDITIONS TO DIET AFTER ONSET OF SYMPTOMS	BLUE UNITS PER 100 GRAMS LIVER	YELLOW UNITS PER 100 GRAMS LIVER	TOTAL BLUE UNITS IN LIVER	TOTAL YELLOW UNITS IN LIVER	B/Y RATIO	FATTY ACIDS PER 100 GRAMS LIVER	WEIGHT OF LIVER	ONSET OF SYMPTOMS (DAYS AFTER DEPANCREATIZATION)	DURATION OF DIABETES	WEIGHT AT OPERATION	WEIGHT AT DEATH	DESCRIPTION OF SYMPTOMS	REMARKS
									gms.		*days*	*kgm.*	*kgm.*		
71	Beef ..... 150 Pancreas ..... 75 Sugar ..... 30 Cracker meal ..... 70 Corn meal ..... 40 Yeast 10	Cystine 0.5 or 0.3 gram for 16 days	2,420	60	10,212	253	40/1	0.8610	422	20	46	6½	4¾	Usual loss of hair, sores and scaling. In addition bilateral superficial keratitis with conjunctivitis and purulent discharge	No improvement after cystine was added. B_2 as yeast
75	*Lecithin* ..... 10 Beef ..... 150 Sugar ..... 30 Cracker meal ..... 70 Corn meal ..... 40 Yeast 5	None	660	200	1,663	504	3 3/1	2.7890	252	66	115	12	6¾	Usual loss of hair, sores and scaliness Loss of hair marked. Purulent discharge from both eyes	Gave birth to 4 pups, 44 days after depancreatization. Symptoms developed 22 days later. B_2 as yeast
86	*Lecithin* ..... 10 Beef ..... 150 Sugar ..... 30 Cracker meal ..... 70 Corn meal ..... 40 Yeast 5	None	None	20	None	75	—	1.8330	378	37	93	10½	7¼	Usual loss of hair, etc.	Died in insulin shock. B_2 as yeast
100	Liver ..... 200 Pancreas ..... 75 Sugar ..... 30 Cracker meal ..... 75 Skim milk ..... 50 Yeast 5	None	44,000	120	188,760	514 8	366/1	5 8275	429	33	41	13	8	Usual loss of hair, sores, etc. Purulent discharge from bladder Purulent conjunctivitis. Submucosal hemorrhages in bladder	Three sources of B_2, raw liver, yeast, skimmed milk powder with no effect on symptoms
106	Liver ..... 200 Pancreas ..... 75 Cracker meal ... 100 Sugar 30	None	220,000	50	1,152,400	379 4	3143 1	3.9085	542	25	81	12½	10½	Usual loss of hair, sores and scaliness	Sources of B_2 raw liver and yeast No effect on symptoms

106	Liver ..... 200 Pancreas ..... 75 Cracker meal ... 100 Sugar ..... 30 Yeast 5	None	220,000	60	1,152,400	379.4	3143/1	3.9985	542	25	81	13½	10½	Usual loss of hair, sores and scaliness	Sources of B_2 raw liver and yeast. No effect on symptoms
96	*Cod Liver oil*, 20 cc. *Lecithin* ..... 10 Beef ..... 150 Sugar ..... 30 Cracker meal ..... 50 Skimmed milk .... 50 Yeast 5	None	22,000	70	51,414	163.6	314/1	3.506	233.7	33	56	12½	7½	Usual loss of hair, sores and scaling. Eyes O.K.	Sources of B_2 skimmed milk powder and yeast
105	*Cod liver oil*, 20 cc. *Lecithin* ..... 10 Beef ..... 200 Sugar ..... 30 Cracker meal ..... 150 Skimmed milk .... 30 Yeast 5	None	9,020	60	43,837	291.6	150/1	7.6845	486	26	77	19	14½	Usual loss of hair, sores and scaling. Deep seated abscess at site of one of injections	B_2 in form of Lederle Liver Ext. every other day for 14 days intramuscularly. Also skimmed milk and yeast
97	Beef ..... 150 Pancreas ..... 75 Sugar ..... 30 Cracker meal ..... 100 Yeast 5	Cod liver oil 30 cc. begun on 55th day given for 24 days	9,900	60	47,866	290	165/1	3.4279	483.5	31	80	12	10½	Usual loss of hair, sores and scaling	Sources of B_2 skimmed milk powder and yeast
104	*Cod liver oil*, 20 cc. Pancreas ..... 75 Beef ..... 150 Sugar ..... 40 Skimmed milk ... 30 Cracker meal ..... 100 Yeast 5	None	22,000	60	136,400	372	366/1	4.5907	620	53	88	15	11½	Usual loss of hair, sores and scaling	Cod liver oil from start with pancreas with no effect on symptoms. Sources of B_2 skimmed milk and yeast
50	Beef ..... 150 Pancreas ..... 75 Sugar ..... 30 Cracker meal ..... 100 Yeast ..... 5 (Skimmed milk for 97 days) .. 30	Cod liver oil 20 cc. and lecithin 10 grams added on 33rd day. Given for 109 days	37,400	70	186,250	367.5	534/1	3.7710	525	26	142	8¼	4¾	Developed usual symptoms which improved 60 days after cod liver oil and lecithin were given	Sources of B_2 yeast and skimmed milk. Dog improved after cod liver oil and lecithin
103	Beef ..... 150 Pancreas ..... 75 Sugar ..... 30 Cracker meal ..... 100 Skimmed milk .... 30 Yeast 5	Cod liver oil 30 cc. and lecithin 10 grams for 20 days. Begun on 81st day	2,420	30	11,737	145.5	81/1	15.825	485	46	102	14½	9	Loss of hair, sores and scaling	Cod liver oil and lecithin had no effect — sources of B_2 yeast and skimmed milk

Skimmed milk in diet refers to skimmed milk powder (Merrall Soule).

but we wish to mention at this time that neither of these had any effect on the loss of hair, sores, or condition of the skin.

PROCEDURE. Adult dogs, mostly females, were used. Prior to depancreatization the animals were kept on a mixed diet for several weeks and only dogs appearing perfectly normal were selected. The diet after depancreatization consisted of raw lean beef, 150 grams, cracker meal 75 to 100 grams, in some dogs corn meal was given in addition, dextrose 30 grams, bone ash 4 grams, yeast (Standard Brands) 5 grams, and 3 grams of salt mixture (11). Any variations are noted in the tables. As indicated in table 1, raw pancreas 75 grams, or lecithin (from egg yolk) 10 grams, or cod liver oil (Mead Johnson) 20 to 30 cc., was added to the diet. Dogs 100 and 106 received 200 grams of raw beef liver in place of lean beef. This is a good source of B_2. Besides yeast all the animals, except 71, 75 and 86, received 30 to 50 grams of skimmed milk powder, which Professor Sherman

TABLE 2

Sources of vitamin A added to diet and effect on vitamin A content of liver

DOG NO.	SOURCE OF VITAMIN A	NO. OF DAYS GIVEN	AMOUNT GIVEN DAILY	BLUE UNITS PER 100 GRAM LIVER	LECITHIN
100	Raw beef liver	41	200 g.	44,000	None
106	Raw beef liver	81	200 g.	220,000	None
97	Cod liver oil	27	30 cc.	9,900	None
104	Cod liver oil	88	20 cc.	22,000	None
96	Cod liver oil	56	20 cc.	22,000	Yes
105	Cod liver oil	77	20 cc.	9,020	Yes
50	Cod liver oil	109	20 cc.	37,400	Yes
103	Cod liver oil	20	30 cc.	2,420	Yes

was kind enough to advise us was a good source of B_2. In addition 105 was given 5 cc. of Lederle's Hepatic Extract intramuscularly every other day for fourteen days. This extract is specific for B_2 deficiency in dogs. (U. S. Public Health Service Reprints nos. 1231 and 1433.) In all, then, four different sources of B_2 were given; yeast, skimmed milk, raw liver and liver extract. Eight of the dogs received at least two sources of the vitamin and two of the dogs, 100 and 105, received three sources of B_2. It would seem that in this way an adequate amount of B_2 had been given. At the end of the experimental period the animals were killed by exsanguination and the livers removed immediately. The Carr and Price modification of the Rosenheim and Drummond antimony trichloride test (12) was used for the determination of the vitamin A content of the livers. The carotene content was also determined on the extracted liver oil. The readings were done in a Lovibond tintometer. The vitamin A is reported in blue units and the carotene in yellow units per 100 grams of whole liver. The total

fatty acids were done by the Mayer and Schaeffer modification of the Kumagawa and Suto method (13).

RESULTS. The symptoms, which we observed originally, occurred in all the animals. The loss of hair occurred first. This usually started over the paws and legs, then about the snout, and at the same time or sooner the hair fell out from the tail. From then on the loss was general, the animals becoming almost completely hairless if the condition was allowed to continue. The skin, in the depilitated areas and over the abdomen became a deep pink and the texture changed, the surface epithelium flaking off readily. Sores appeared first over the joints, later at the base of the ears, the sides of the face and the buttock. The stools showed no gross evidence of a deranged fat metabolism. In some of the animals the bulk was greatly increased and the consistency soft, in others the stools remained firm. During the later stages, however, they were usually blood tinged and this was often followed by a diarrhea.

None of the sources of B_2 had any effect on the symptoms. Nor did cystine 0.5 to 0.3 gram daily for 16 days given to 71 in any way prevent the loss of hair. Lecithin given from the time the animals were depancreatized, as in 75 and 86, did not prevent the symptoms. Cod liver oil given alone or with lecithin neither prevented nor improved the symptoms (dogs 96, 97, 103, 104, 105) except in dog 50. This animal was given lecithin and cod liver oil after the symptoms appeared. The sores healed, in some places the hair grew back and the general condition of the dog improved. This was the only one of the entire group that showed improvement.

Of the 8 animals receiving a source of vitamin A, 5 had large amounts in their livers. Of the 3 in whom the concentration of the vitamin was low, two, 97 and 103, had been on cod liver oil for only 27 and 20 days respectively. The third, 105, when killed was found to have a large abscess, which may have depleted the vitamin stores. The greatest amounts of the vitamin were in the dogs, 100 and 106, receiving raw beef liver. In the dogs receiving cod liver oil, the blue unitage was highest in the animals that had been receiving it for the longest period of time. This was dog 50, that had also received lecithin and was the one that showed some signs of improvement after 60 days on cod liver oil. The crude egg yolk lecithin which we used contained no vitamin A as such when extracted by the Carr and Price method, but did contain small amounts of carotene. The two dogs receiving lecithin alone had very small amounts of vitamin A in their livers, so that it does not seem likely that the lecithin provided a sufficient amount of carotene to serve as a source of vitamin A. In the animals studied previously (1) carotene given in oil was apparently not converted to vitamin A. It may have been that the amounts were not great enough, although the liver of a non-diabetic A deficient dog, fed the same amount of carotene, contained large amounts of the vitamin.

The total fatty acids were higher in the animals on vitamin A. One would expect cod liver oil to increase the amount of fat in the liver. We are at present studying the nature of these fats and in our next group of animals hope to report the degree of unsaturation of the liver phospholipids and neutral fats. Sinclair (14) found "a definite quantitative relationship between the amount of cod liver oil ingested per day and the iodine number of the phospholipid fatty acids" in rats. The effect was more pronounced on the liver phospholipids than on those of the skeletal muscle. The amounts of fat "sufficient to produce a marked increase in the level of unsaturation of the phospholipids" had no apparent effect on the degree of unsaturation of the neutral fat.

Discussion. The fact that the symptoms observed in depancreatized dogs are not cured by the administration of vitamin A, even though large amounts of the vitamin are found in the livers of such animals, suggests that a lack of this vitamin is not responsible for the symptoms. On the other hand there might exist some interference with the utilization of the vitamin. If we argue that the symptoms are not due to a deficiency of vitamin A, what other factors might be responsible? B_2, a deficiency of which in rats produces loss of hair, is we think ruled out. In this group of dogs it was given in sufficient amounts and in normal dogs studied in this laboratory (unpublished experiments) we have not found that B_2 had any effect on these symptoms. The other possible cause is some disturbance in the metabolism of the fatty acids. This might be due to a lack of absorption of certain essential fatty acids, which Burr and Burr (5) and Evans and Lepkovsky (6) feel cannot be synthesized by the animal organism.

The possibility of an interference with the utilization of the vitamin, even though present in the liver, might also be related to a faulty fat metabolism. Sinclair (14) suggests that in rats on a low fat diet there may be a deficient utilization of the fat soluble vitamins. The source of fat in the diets fed our depancreatized dogs was that contained in the raw lean beef. A depancreatized dog loses the external secretion of the pancreas and although gross disturbances of fat metabolism may be controlled by feeding raw pancreas, it is possible that the absorption of unsaturated fatty acids or their utilization might not be controlled by such means. We can only surmise on this at the moment, but we hope that future experiments will yield further information on these points.

We must acknowledge, in the light of the evidence presented by this second group of animals, that the symptoms occurring in depancreatized dogs are apparently not due to a lack of vitamin A in the livers of these animals.

SUMMARY

Symptoms consisting of loss of hair, sores and scaliness of the skin occurred in 11 dogs after depancreatization.

Cystine had no effect on these symptoms.

Four sources of B_2, yeast, raw liver, skimmed milk powder and liver extract, did not improve the symptoms.

Lecithin, 10 grams daily, given from the time the animals were depancreatized, did not prevent the symptoms.

Cod liver oil given alone and with lecithin did not prevent or cure the symptoms.

The livers of 5 of the 8 dogs receiving a source of vitamin A, contained large amounts of the vitamin.

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RELATION OF THE OXYGEN AND NITROGEN CONTENT OF CEREBROSPINAL FLUID TO BAROMETRIC PRESSURE

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The purpose of this investigation was to determine the relation between the oxygen and nitrogen content of the cerebrospinal fluid of dogs and the barometric pressure of the atmosphere which surrounded them.

Cerebrospinal fluid was chosen as being more suitable than blood because it does not contain the complicating factor of hemoglobin.

The dogs used as experimental animals were chosen both for size and condition, the larger dogs being more desirable because of the greater amount of spinal fluid which could be withdrawn without injury. Fluid was not withdrawn oftener than every three days. All dogs were kept under pressure at least ten hours before the withdrawal of the fluid.

APPARATUS AND EQUIPMENT. The equipment for the analysis consisted of two Van Slyke manometric blood gas apparatus, modified Hempel pipettes for storing reagents and Ostwald-Van Slyke pipettes, fitted with rubber tips and stopcocks for delivery of the samples. The manometer tubes of the gas analysis apparatus were equipped with vernier scales, such as are used on the better barometers. These scales greatly reduced the error in reading the meniscus of the mercury in the tube.

The compression chamber, or "tank," in which the dogs were kept throughout the entire experiment consisted of a cylindrical enclosure, 35 feet long by 16 feet in diameter, constructed of $\frac{1}{2}$ inch steel boiler plate. It was equipped with hermetically sealed doors and an air lock.

When operating at above atmospheric pressure, the tank was supplied with air which, after being compressed, was conditioned by means of refrigeration to approximately 65 per cent relative humidity at 72°F. It was then diverted from other tanks at the rate of approximately two hundred cubic feet of free air per minute. A large compressor was used, the cylinder of which was lubricated with glycerin. We regard the lubricant of the cylinder as important.

Whenever the lock was used to enter or leave the tank while the pressure was on, the air used to fill the lock was taken from another tank, so that a drop in temperature or pressure in the experimental tank was avoided.

TECHNIQUE. The fluid was obtained aseptically by means of a cisternal puncture, the site being anesthetised by a 1 per cent aqueous solution of novocain. The first few drops of fluid to emerge were discarded. An oiled 10 cc. syringe was then fastened to the needle, and approximately 6 cc. were withdrawn. Immediately after withdrawal, the fluid was placed

TABLE 1

Oxygen and nitrogen content of cerebrospinal fluid of dogs at atmospheric pressure and plus thirty pounds pressure

DOG	ATMOSPHERIC PRESSURE		ATMOSPHERIC PRESSURE PLUS THIRTY POUNDS†	
	O_2	N_2	O_2	N_2
	volume per cent	*volume per cent*	*volume per cent*	*volume per cent*
D42	0.20*	0.96	0.42	2.72
D43	0.18	0.90	0.40	2.68
D44	0.27	0.97	0.55	2.50
D45	0.16	0.90	0.41	2.60
D47	0.19	0.93	0.51	2.81
D48	0.18	0.92	0.47	2.62
D49	0.20	0.90	0.47	2.66
D51	0.17	0.89	0.35	2.83
D52	0.20	0.90	0.43	2.70
D53	0.25	0.90	0.44	2.41
D54	0.21	0.90	0.47	2.75
D57	0.20	0.86	0.44	2.63
D58	0.24	0.87	0.44	2.71
D59	0.19	0.92	0.49	2.83
D60	0.18	0.90	0.40	2.71
D62	0.18	0.94	0.47	2.83
Average.....	0.20	0.91	0.44	2.69

	CALCULATED PER CENT INCREASE ACCORDING TO HENRY'S LAW	ACTUAL PER CENT INCREASE
Average per cent O_2 increase.....	200.0	120.0
Average per cent N_2 increase.....	200.0	196.7

* Each figure in this chart represents the average of two analyses done on the same fluid, all duplicate determinations included, checking each other within 0.05 volume per cent.

† Variation from 29 to 31 pounds.

in a small glass test tube under oil. From this it was transferred as soon as possible to two 2 ml. Ostwald-Van Slyke pipettes, and then to the chambers of two Van Slyke gas analysis apparatus. Duplicate readings were thus made in all cases studied.

The technique used in the analysis was that recommended by Van Slyke

and Neill (1924) for analysis of whole blood, with the exception that 8 cc. of a $\frac{1}{20}$ normal lactic acid were used instead of the same amount of acid saponin ferricyanide solution.

Lactic acid alone was used, because in the case of spinal fluid, no saponin or ferricyanide was necessary, there being no erythrocytes or oxyhemoglobin to contend with. The carbon dioxide was removed by a sodium hydroxide solution and the oxygen absorbed by sodium hydrosulphite with the catalyst, sodium anthraquinone-beta-sulfonate. While under pressure above atmospheric pressure, the task of getting the fliud into the chamber of the machine was greatly increased. The fluid could not be brought down to atmospheric pressure in an ordinary container, because the decrease in pressure would cause a portion of the dissolved gases to escape. This problem was solved by taking the entire machine into the tank and putting the fluid into it here under the increased pressure. But it was impossible to perform the analysis in the tank under this pressure because the height of the column of mercury necessary to produce a vacuum would be too great for practical use. The greatest source of error in the analysis is that of bringing the fluid in the chamber to the five-tenths cubic centimeter mark. This error was calculated by Van Slyke (1924) as being less than five-hundredths of one volume per cent in a 2 cc. sample. Whenever the results of the two apparatus did not check within this limit, they were discarded.

DISCUSSION. The results of these experiments show quite clearly that the oxygen tension of the spinal fluid does increase with the barometric pressure but, however, not according to Henry's law. The nitrogen tension, on the other hand, varies nearly, if not exactly according to Henry's law, directly with the barometric pressure.

The difference in behavior of these two gases can probably be explained by the fact that nitrogen takes very little, if any, part in the body metabolism, whereas oxygen is continually being used.

SUMMARY. 1. The oxygen content of normal cerebrospinal fluid of dogs varies from 0.16 to 0.25 vol. per cent at atmospheric pressure.

2. The nitrogen content of normal cerebrospinal fluid of dogs varies from 0.86 to 0.97 vol. per cent at atmospheric pressure.

3. The oxygen content does not increase according to Henry's law at plus 30 pounds pressure. It does, however, increase 120 per cent.

4. The nitrogen content increases very nearly, if not exactly according to Henry's law, at plus 30 pounds pressure, the actual increase being 197 per cent, as compared to the calculated 200 per cent.

CONCLUSION

Roughly stated, the oxygen content of cerebrospinal fluid is a little more than doubled, and the nitrogen content is trebled, when the barometric

pressure is increased from one atmosphere to three atmospheres (30 pounds gauge).

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THE ACTION OF HISTAMINASE ON THE GASTRIC SECRETORY RESPONSE TO HISTAMINE AND TO A MEAL

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It has been shown (Sacks, Ivy, Burgess and Vandolah, 1932) that if a mixture of histaminase and "gastrin," or of histaminase and histamine, is incubated *in vitro* and then injected subcutaneously, gastric secretion is not stimulated. It appeared to be desirable for certain theoretical and practical considerations to ascertain if histaminase (Best, 1929) administered parenterally might prevent or inhibit the gastric secretory response to histamine or to a meal. Although the failure of histaminase to do so might be predicted on the basis of a consideration of some of the *in vitro* studies of Best and McHenry (1930), it was considered important to answer the question by direct experimentation.

METHODS. A quantity of "histaminase powder" was prepared from the kidneys of dogs according to the method of Best and McHenry (1930). It was found that 100 mgm. of this powder in 10 cc. of distilled water destroyed 1.0 mgm. of histamine (ergamine acid phosphate) when incubated for 24 hours, the gastric secretory response being employed to test for the presence of active histamine. The digestion was conducted aseptically by passing the mixture prior to incubation through a Berkefeld filter into a sterile container. Repeated assays on the "powder" during the course of the experiments showed it to be stable. Non-refractory Pavlov pouch dogs were used. Each type of experiment was repeated five or more times.

RESULTS. *a.* It was found that the "histaminase powder" itself when injected subcutaneously or slowly intravenously in doses of from 100 to 200 mgm. did not stimulate gastric secretion.

b. When either 100 or 200 mgm. of the "histaminase powder" in 10 cc. of water were mixed with 1.0 mgm. of histamine and passed through a Berkefeld filter and then immediately injected subcutaneously, a typical gastric secretory response resulted. This showed that a significant destruction of the histamine by the histaminase at the site of injection did not occur.

c. When 500 mgm. of the "histaminase powder" in 260 cc. of normal

saline solution were injected intravenously during a period of 1.5 hours, the gastric secretory response to 1.0 mgm. of histamine, injected subcutaneously 5 minutes after the start of the intravenous injection of the histaminase, was not inhibited. In control experiments, it was found that the intravenous injection of 500 mgm. of the "histaminase powder" in 260 cc. of normal saline solution during a period of 1.5 hours did not stimulate gastric secretion. Also, when 3500 mgm. of the "histaminase powder" were injected intravenously during a period of an hour and 45 minutes, the gastric secretory response to 1.0 mgm. of histamine, injected subcutaneously 15 minutes after the start of the intravenous injection of the histaminase, was not inhibited.

d. The intravenous injection of "histaminase powder" as in the foregoing experiments failed to diminish the gastric secretory response to a test meal of meat and milk. Similarly a negative result was obtained when the "histaminase powder" was fed with the test meal.

Discussion. Best and McHenry (1930) found in *in vitro* studies that at least a period of 10 hours incubation is required for 200 mgm. of the "histaminase powder" to destroy 1.0 mgm. of histamine. Hence, the failure of histaminase, when mixed with histamine and injected at once subcutaneously, to diminish the gastric secretory response to histamine is without doubt due to the fact that histamine is absorbed more rapidly than it is inactivated by histaminase. The time factor also probably accounts for the failure to obtain inactivation of histamine in the experiments in which histaminase was injected intravenously.

CONCLUSION

Histaminase administered parenterally in doses more than sufficient to inactivate *in vitro* in from 10 to 24 hours the amount of histamine employed, does not inhibit the gastric secretory response produced by histamine. Histaminase in the doses employed does not inhibit the gastric secretory response to a meal.

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PHYSIOLOGICAL EFFECTS OF HIGH FREQUENCY CURRENT

V. The Non-protein Nitrogen Partition and the Secretion of Urine in Anesthetized Dogs[1]

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It is believed that acute hyperthermia induced by physical means does not exert any measurable influence upon protein metabolism (Graham and Poulton, 1912). This is not true of certain infective fevers such as typhoid (Shaffer and Coleman, 1909). Having observed that the concentration of non-protein nitrogen (n.p.n.) of dog blood is greatly increased during hyperthermia induced by high frequency current (Nasset, Bishop and Warren, 1931) it was decided to investigate this problem further with a view to ascertaining whether the chief fractions of the n.p.n. were concentrated in a parallel fashion. Knudson and Schaible (1931) reported n.p.n. partition in the blood of 7 unanesthetized dogs treated with "ultra-high frequency" current. Blood urea was determined in 5 experiments before and after exposure. In one of these the ratio of urea nitrogen to n.p.n. did not change, in two it was increased, and in the remaining two it was decreased. The rise in n.p.n. was attributed to increased metabolism, concentration of the blood, and oliguria. Perkins (1931) made a similar study on 39 *dementia paralytica* patients who were treated with diathermy. It is apparent from his results that, on the average, the ratio of urea nitrogen to n.p.n. was slightly increased and that the urea remained above normal longer than did n.p.n. These investigators did not report any determinations of the nitrogen output in the urine.

Methods. Dogs (12–25 kgm.) anesthetized with sodium amytal served as experimental animals. They were fasted 40 hours or more before being used for experiment. The diet consisted of hospital kitchen scraps, usually rich in carbohydrate. The following chemical procedures were used: precipitation of blood proteins (Haden, 1923), n.p.n. (Folin and Wu, 1919), sugar (Benedict, 1928), urea (Leiboff and Kahn, 1929), creatine plus creatinine (Hawk and Bergeim, 1931), amino acids (Folin, 1922). A blood specimen was obtained from the saphenous or external jugular vein

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just preceding anesthesia, another just prior to the application of the current (10^6 cycles / sec. body electrodes), and then at intervals during the rise of body temperature. Urine was collected from ureteral cannulae in hourly periods, the volume noted, and total nitrogen determined by the macro Kjeldahl method. In order to check further the effect of amytal on sugar and n.p.n., these blood constituents were determined in a series of 12 dogs used by the second year medical class for sacrifice experiments in the physiological laboratory. Blood samples were obtained before anesthesia and at convenient intervals thereafter ($2\frac{1}{4}$–$5\frac{1}{2}$ hrs.).

Results. The results are presented in condensed form in tables 1 to 4. In table 1 we averaged the results obtained in 16 experiments. Individual experiments ran from 4 to 7 hours. For purposes of tabulation we chose to group the analytical results according to their incidence in four different ranges of rectal temperature. The first group comprises all of the results obtained with the rectal temperature 38.5°C. or below. This group was made to include all determinations made before any heating was begun and represents the "normal" state. A rectal temperature of about 42°C. appears to be rather a critical one in this type of experiment. After attaining this temperature the organism is unable to maintain conditions which are typical of it in the normal state. For example, respiration, n.p.n., blood sugar, hematocrit, and hemoglobin saturation of venous blood are all rather markedly altered (Nasset et al., 1931; Nasset, 1932a; Nasset, 1932b). We therefore arranged the remaining results so that the second group fell between the "normal" and the "critical" temperatures, namely, at about 40°C.; the third at about 42°C., and the fourth at 44°C. The last group includes all data obtained above 42.5°C. We thus have 4 temperature groups the averages of which are separated successively by about 2°C.

Since creatinine was such a small fraction of the n.p.n. and exhibited no gross changes in concentration, its determination was discontinued after the first nine experiments. We were aware that the uric acid concentration of the blood of most dogs is very low, but it was thought that the inclusion of the estimation of this substance might afford some information, together with creatinine, concerning the endogenous protein metabolism. It failed to show anything but relative constancy and was discontinued after six experiments.

The controls for the n.p.n. partition are presented in table 2. In these experiments no current was applied to the animal and the rectal temperature remained fairly constant. From 4 to 6 samples of blood were analyzed in each control. The average for each series appears in the table. These experiments were continued for approximately the same length of time as were those just described (table 1).

Eleven additional experiments were carried out in which the ureters were

cannulated and the urine sampled at intervals of one hour. In six of these experiments the blood was sampled simultaneously and analyzed for n.p.n., urea, and sugar. The results of these experiments (table 3) were grouped in the same manner as those in table 1. The urine secretion controls appear in table 4. For the purpose of giving a clearer idea of the course of a single experiment, a fairly typical one is shown graphically in figure 1.

TABLE 1

Average NPN partition and sugar in the blood of anesthetized dogs treated with high frequency current (16 experiments)

RANGE OF RECTAL TEMPERATURE	AVERAGE RECTAL TEMPERATURE	MILLIGRAMS PER 100 CC.						
		Sugar	NPN	Urea N	Amino acid N	Creatinine N	Uric acid N	Undetermined N
°C.	°C.							
<38.6	37.4	76	27.5	13.4(48.7%)*	6.4(23.3%)	0.64(2.3%)	0.27(1.0%)	6.8(24.7%)
38.6–41.0	40.0	78	28.2	13.7(48.6%)	6.5(23.0%)	0.71(2.5%)	0.34(1.2%)	6.9(24.7%)
41.1–42.5	41.8	71	32.6	15.5(47.5%)	6.9(21.2%)	0.71(2.2%)	0.31(1.0%)	9.1(28.1%)
>42.5	44.0	55	38.9	19.3(49.6%)	7.8(20.1%)	0.76(2.0%)	0.34(0.9%)	10.7(27.4%)

* Percentage of the total NPN.

TABLE 2

Sugar and NPN partition controls

EXPERIMENT NUMBER	AVERAGE RECTAL TEMPERATURE	MILLIGRAMS PER 100 CC.						
		Sugar	NPN	Urea N	Amino acid N	Creatinine N	Uric acid N	Undetermined N
	°C.							
26	38.2	57	33.8	18.0	6.8	0.74	0.29	7.2
30	38.4	94	28.7	14.4	6.6	—	—	—
39	38.1	65	29.4	12.5	6.3	—	—	—
40	38.2	64	27.9	12.9	5.8	—	—	—
Average..	38.2	70	30.0	14.5(48.3%)	6.4(21.3%)	0.74(2.5%)	0.29(1.0%)	7.2(24.0%)

The analytical results from the dogs used for student experiments simply confirm our previous findings concerning the effect of amytal on blood n.p.n. and sugar. The average values before anesthesia were: 31.4, 14.9, and 67 mgm. per 100 cc. respectively for n.p.n., urea nitrogen, and sugar; after anesthesia ($2\frac{1}{4}$–$5\frac{1}{2}$ hrs.): 31.8, 14.8, and 73 mgm. per 100 cc. respectively.

DISCUSSION. A very striking feature of the data in table 1 is that throughout the whole temperature range from 37.4° to 44.0°C. the urea nitrogen remained nearly a constant percentage of the total n.p.n. In fact, the relative concentrations of all four of the fractions which were determined maintained approximate constancy. The slight fall in the percentage of amino acid and creatinine nitrogen accounts for the rise in the percentage of undetermined nitrogen. On comparison of these results with those of the controls it is seen that as regards relative concentration of the various fractions of the n.p.n. there has been no significant change.

TABLE 3

Urine secretion as related to blood NPN and urea in hyperthermia (11 experiments)

TEMPERATURE RANGE	AVERAGE RECTAL TEMPERATURE	URINE VOLUME	TOTAL URINE N	CONCENTRATION URINE N	BLOOD NPN	BLOOD UREA N
	°C.	cc./hr.	mgm./hr.	mgm./cc.	mgm./100 cc.	mgm./100 cc.
<38.6	37.6	7.2	139	19	29	13.0(44.8%)
38.6–41.0	40.1	6.8	175	26	30	13.6(45.3%)
41.1–42.5	42.0	4.8	139	29	36	15.4(42.8%)
>42.5	43.0	1.5	43	29	43	17.6(40.9%)

TABLE 4

Urine secretion controls

EXPERIMENT NUMBER	AVERAGE RECTAL TEMPERATURE	URINE VOLUME	TOTAL URINE N	CONCENTRATION URINE N	BLOOD NPN	BLOOD UREA N
	°C.	cc./hr.	mgm./hr.	mgm./cc.	mgm./100 cc.	mgm./100 cc.
54	37.7	9.4	122	15	31	14.1
72	37.8	8.7	299	36	—	—
74	37.3	5.6	195	35	—	—
Average..	37.6	7.9	205	29	31	14.1(45.5%)

Judging from the results obtained in the urine secretion experiments (table 3) it is reasonable to assume that the animals attaining a rectal temperature of 42–44°C. (table 1) would be anuric or very nearly so. Since urea nitrogen constitutes about 80 per cent of the total nitrogen of the urine, blocking its path of escape should lead to its rapid accumulation in the body. It seems strange with the nitrogen retention which must occur under these conditions that the relative concentration of urea is not greatly increased. Bollman, Mann and Magath (1924) found that, in the nephrectomized dog, blood urea accumulated at a uniform rate so that the concentration was increased 50 per cent in about two hours and doubled in four hours. According to Marshall and Davis (1914) urea is very readily

and rather uniformly distributed throughout approximately 90 per cent of the tissues of the body. They injected varying doses and found that in a few minutes 90 per cent of the urea had lodged in tissues other than the blood. If these two facts, i.e., the rapid accumulation of urea in nephrectomized animals and its ready diffusibility to all tissues, hold for oliguria or anuria such as manifestly obtained in our experiments, then the rate of urea formation must have been somewhat accelerated. For example, in experiment 50 (fig. 1) the urea nitrogen of the blood remained constant until after the fourth blood specimen was taken. The total output of urinary nitrogen for the 3 hours just preceding this was 571 mgm. or 457

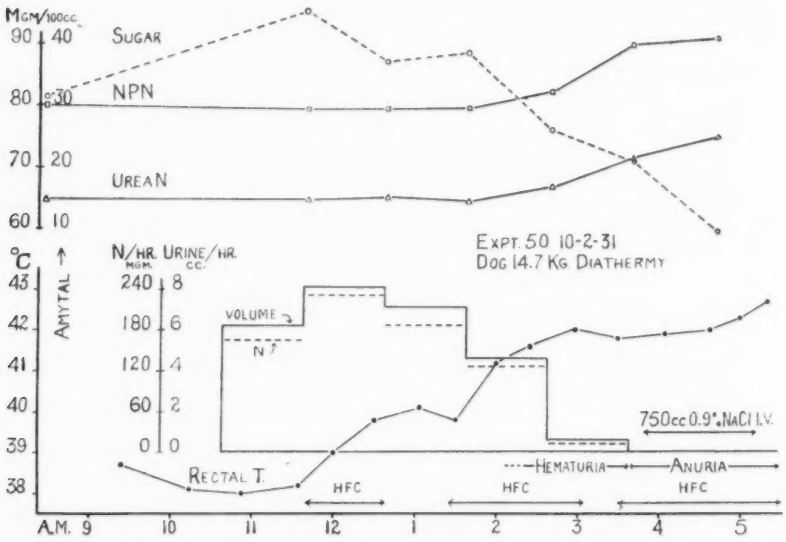

Fig. 1

mgm. of urea nitrogen, assuming the latter to be 80 per cent of the total. The amount excreted must have been very near to the production of urea during this period because its concentration in the blood remained unaltered. For the last three hours of the experiment the total nitrogen excretion was 141 mgm. corresponding to 113 mgm. of urea nitrogen. During this same interval of time the urea nitrogen of the blood rose 10.3 mgm. per 100 cc. (from 14.3 to 24.6). This dog weighed 14.7 kgm. and to raise the concentration to 90 per cent of his whole body by the amount indicated in the blood would have required the production and retention of 1359 mgm. of urea nitrogen. Add to this the 141 mgm. excreted and we have a total of 1500 mgm. This represents a three-fold increase in protein

metabolism. Although increased protein metabolism is not generally associated with hyperthermia induced by physical means, it appears to be highly probable when the body temperature is elevated to 42 or 44°C.

However, we do not believe that the extent of protein breakdown is accurately indicated by the above calculation but rather that the concentration of urea in the blood is not a reliable measure of tissue urea when the rate of its elimination from the body is changing rapidly. In one of the animals (G-4 wt. 16.6 kgm.) of Bollman, Mann and Magath the blood urea nitrogen after nephrectomy increased 9.85 mgm. per 100 cc. in four and one-half hours. To attain this increase in 90 per cent of the body would have required the production in this time of 1468 mgm. of urea nitrogen—a rate of 326 mgm. per hour. This seems rather too much for a dog of this size in the post-absorptive state. The data from another of their dogs (C-671 wt. 13.2 kgm.) with kidneys intact show that, after removal of the liver, 685 mgm. of urea and ammonia nitrogen were excreted in twelve hours while the blood urea nitrogen fell from 9.0 to 2.15 mgm. per 100 cc. To remove a corresponding amount from 90 per cent of the body would have required the excretion of 815 mgm. Although these calculations must be approximations only they demonstrate quite well the point which we made above, namely, that the movement of urea in or out of tissues entails a certain amount of lag. In the case of the nephrectomized dog where the avenue of escape for the urea is closed the concentration of blood urea increases more rapidly than the other tissues because it is the first to receive it from the liver. On the other hand, in the dehepatized dog when urea is no longer being formed the blood, being the first tissue to be cleared of urea, is likely to show the greatest diminution in urea content. This course of events is the natural consequence of opposite concentration gradients which must obtain between blood and other tissues in these two experimental conditions. The intravenous injection of urea as employed by Marshall and Davis may alter the conditions somewhat but their data are capable of a similar interpretation. For example, one of their dogs (D4 wt. 4 kgm.) was given 20 grams of urea intravenously and killed 4 hours later. Liver, muscle, spleen, heart, and brain as well as blood were analyzed for urea. The results indicate that urea, on the average, was only 77 per cent as concentrated in the solid tissues as in the blood. Assuming that our dog 50 (fig. 1) contained 1 liter of blood, and that the above findings of Marshall and Davis can be applied, it was calculated that the retention of about 1 gram of urea nitrogen would have been required to produce the observed change in blood urea. This would represent a doubling of the normal protein metabolism. The same calculation based upon average figures (table 3) indicates that the required protein metabolism is 2.4 times as great as normal. It thus appears more than likely that in this type of experiment the protein metabolism runs

approximately parallel to the total respiratory metabolism (Nasset et al., 1931; Nasset, 1932a).

The discussion concerning urea has indicated that protein metabolism is augmented in severe hyperthermia. A confirmation of this is scarcely to be found in the data concerning amino acid, creatinine, and uric acid nitrogen. Over the whole temperature range studied (table 1) these fractions of the n.p.n. were increased 22, 19 and 26 per cent respectively, whereas total n.p.n., urea nitrogen, and undetermined nitrogen increased 42, 44 and 57 per cent respectively. It is known that in this type of experiment the hematocrit values may be very greatly increased (Nasset, 1932b). Since the amino acids are found in much greater amount in cells than in plasma (R. Martens, 1928) it is likely that the change in amino acid nitrogen may be almost wholly associated with the change in hematocrit. Okada and Hayashi (1922) found that ligation of the ureters of dogs resulted in a marked rise of the amount of amino acid in the blood. This result still requires explanation. It is not known whether anuria produced by high body temperature might have a similar result; our data do not support such an hypothesis. The creatinine and uric acid being about evenly distributed between cells and plasma would be unaffected by any change in cell volume, but diminished excretion in the urine and anhydremia (Peters and Nasset, 1933) would probably account for the change in concentration of these compounds. Certainly they show no evidence of marked change in endogenous protein metabolism. The indications are, therefore, that any increase in protein breakdown is at the expense of deposit protein.

The reasons for anuria at high temperature are not entirely clear. Several suggest themselves such as: dehydration, lowered blood pressure, and actual damage to the kidney parenchyma. We know that the animal is rather severely dehydrated under our experimental conditions (Peters and Nasset, 1933). The blood pressure change is scarcely a factor because it has been observed to remain at a relatively high level until just prior to exitus. The hypothesis that there is some damage to the functional tissue of the kidney is confirmed by microscopic examination. There is some early histo-pathological change noted as cloudy swelling in the epithelium of the convoluted tubules. It is perhaps impossible to evaluate this change on a functional basis since it is well known that there may be great functional disorder without significant histological change. Further confirmation is afforded by the fact that once anuric the kidney fails to respond to measures which usually call forth a copious flow of urine. In several experiments after the cessation of the flow of urine, caffeine citrate was injected both before and after the injection of large volumes of normal saline without reëstablishing the flow. As much as 750 cc. of saline was used for a single injection (fig. 1). In a few instances hematuria was noted in the terminal stages of the experiment. We feel, therefore, that dehydration

and renal damage are at least two factors in diminishing the flow of urine in these experiments.

SUMMARY

1. In hyperthermia induced by high frequency current the urea, amino acid, creatinine, and uric acid nitrogen fractions of the blood n.p.n. maintain essentially the same relative concentrations up to a rectal temperature of 42 to 44°C.

2. There is some evidence that at these high temperatures protein metabolism, as judged from urea production, may be doubled. There is no evidence of a disturbance to the endogenous protein metabolism.

3. Oliguria and anuria are almost invariable sequelae to hyperthermia carried to 42°C. or higher. It is suggested that dehydration and damage to the renal parenchyma are important factors in the loss of kidney function.

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DOES THE VENTRICLE EXERT A SUCTION ACTION IN DIASTOLE?

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Although this question has been the subject of much discussion and theoretical argument in the past, valid experimental work on the question is very meagre. Katz (1930) has critically reviewed previous work on the subject and drawn attention to the necessity for a more reliable type of evidence than hitherto adduced. He has further attempted to obtain direct experimental evidence on the matter from which (supported by the data of the volume curves of the ventricles) he has drawn the conclusion that "the ventricle not only can but does exert a sucking action in the intact animal."

If this be true, an important aspect of the mechanics of the cardiac cycle has been clarified. If however the evidence be fallacious, there is every possibility that the stated conclusion may gain currency in physiological thought and lead to error and confusion in matters related to cardiodynamics.

It is the object of the present communication to show that the evidence adduced by Katz involves a fallacy, and that the character of the records obtained were necessarily produced by the hydrodynamics of his set-up, and were not determined by the mechanics of cardiac activity.

In order to relate the present experiments to those of Katz, it is necessary to consider in some detail the precise physical arrangements obtaining therein.

The general argument may be summarised as follows. A system is so arranged that when the ventricle (of the surviving turtle's heart) contracts, fluid is forced upward via the aorta through connecting tubes, into a reservoir, and the concomitant increase of pressure is recorded by an optical manometer inserted into the ventricular cavity through the A. V. ring. Further, when the ventricle relaxes, a drop of the intraventricular pressure curve below the zero line[2] is taken as evidence that a suction action is exerted by the ventricle in diastole.

[1] Traveling Fellow of the Rockefeller Foundation.

[2] Zero determined statically after the ventricle has ceased to beat.

As Katz appropriately remarks, "a narrow constriction[3] in the tubes through which the ventricle fills, will exaggerate any pressure variations which activity of the ventricle might produce, and so make it more appreciable."

While this is true, it is here submitted that the apparent negative pressure registered in the records, is not necessarily produced by any suction action on the part of the ventricle, but is an inevitable hydrodynamic consequence of placing the constriction there.

The detailed criticism of the apparatus may be stated by considering in stages the sequence of events during the complete cardiac cycle.

Stage 1. Just prior to ventricular systole, the pressure obtaining at the level of the manometer membrane is represented by the level portion of the recorded tracing (*P* in fig. 2 of Katz's paper). This level represents the *static pressure* operating on the membrane, due to the height of the fluid level in the reservoir, over the height of the membrane. Now it is deducible from the records themselves, that this level must necessarily be much higher than indicated in the diagram, in order to produce the curves shown, and that the diagram has been reduced vertically *in this respect*, for convenience of reproduction.

Stage 2. With the onset of systole, considerable resistance is offered to the flow of saline through the constriction so that the pressure rises abruptly. (See fig. 2 of Katz's paper.)

Stage 3. When systole ceases, the flow through the constriction is reversed, but, *inasmuch as the constriction is present, the full hydrostatic pressure of the fluid extending above to the level in the reservoir, is unable to operate on the manometer membrane, and cannot do so until static conditions are once more resumed.*

In the meantime, therefore, the pressure in the fluid system below the constriction is much reduced,[4] and the record must inevitably show a fall below the base line, as is actually seen in figure 2 of Katz's paper. This contention receives further support from the statement in Katz's paper that, "the reduction of pressure below the zero level of the system was found to be greatest when this zero level was highest, and when the ventricular activity was most vigorous."

This would also be expected from the hydrodynamics of the system. For in the first place, the higher the zero level, the greater the height of

[3] I.e., in the connecting tubes from ventricle to reservoir.

[4] This effect of the constriction in shielding the manometer system from the full potential pressure, under dynamic conditions, is analogous to the effect of the arterioles in producing a sharp gradient of pressure between the terminal arterial system and the capillaries. This is well illustrated in a diagram published by Landis to express such gradients of blood pressure, which he has established by direct experiment.

the fluid level in the reservoir above the manometer membrane, and therefore the greater the shielding of the membrane from the full hydrostatic pressure, when flow through the constriction is proceeding backwards into the ventricle in diastole.

In the second place a more vigorous contraction would force more fluid into the reservoir, and more time be required for it to return by gravity through the constriction, so allowing more scope for the negative pressure to be developed.

Although these considerations seemed clear enough to invalidate the conclusions of Katz, it appeared worth while to obtain direct experimental evidence in support of the present criticism. An effective proof of the present contention would be realised if a system could be set up, similar

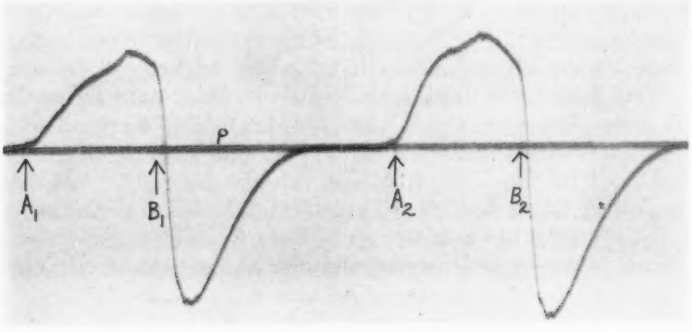

Fig. 1. P = base line of pressure.

At A_1 and A_2 plus external air pressure applied to "artificial ventricle."

At B_1 and B_2 external air pressure released, causing a rapid decrease of "intraventricular" pressure well below the base line in imitation of "ventricular suction" of Katz's records.

in all dynamic essentials to that of Katz, but in which the heart could be replaced by a variable capacity, which could not possibly exert any suction, and then to cause the system to function in such a way as to produce similar records to those published by him as demonstrating suction.

It was therefore arranged to set up such an essentially similar system. The reservoir used in place of the ventricle was a small rubber balloon, which had been immersed (when just moderately distended with water) several times in a solution of collodion, to provide an inextensible covering without interfering with flexibility.

This balloon was enclosed in a similar fashion to the ventricle in Katz's experiments. Inlet and outlet tubes and taps were provided, so that it was possible by turning one tap, to apply quickly positive air pressure to

the balloon, to lend it, as it were, a substitute for contractile force, in imitation of systole, and then by turning a second tap, to secure a rapid removal of external pressure by the quick escape of the compressing air. A typical record so obtained is shown in figure 1.

Since this record shows the same essential features as those obtained by Katz with the turtle's ventricle, the conclusion is drawn that his experimental evidence involves a fallacy and cannot therefore be accepted to decide the problem attacked.

Further evidence is, however, adduced by Katz, which it is now necessary to consider. This evidence consists of inferences drawn from curves, published by Wiggers, of pressure and volume changes of the heart during the cardiac cycle. (Vide fig. 6 of Katz's paper.)

The argument developed by Katz from these curves is stated by him as follows:

> During rapid inflow (VI to VII) the pressure drops and the volume increases; . . . . Were filling entirely due to a passive distention, during this phase, one would expect to find an increase in both the volume and pressure, as is the case during distention of any elastic body and is actually the case during diastasis and early auricular activity. The fact that the pressure drops during filling indicates that the ventricle is relaxing at a faster rate than it can fill. Such a state of affairs is inconceivable if the filling were entirely passive during this phase.

The criticism offered is that such a state of affairs is not incompatible with passive filling, but that the opposite trend of the volume and pressure curves is merely an expression of the fact that *between VI and VII the decreasing tension of the ventricle wall in late relaxation has fallen below the prevailing tension in the walls of the atrium* so that blood passes from this chamber into the ventricle with a *simultaneous drop of pressure in both*.

The writer believes that Katz has devised the best experimental attack on the problem to date, and that his method suitably modified to avoid the hydrodynamic artifact is really capable of deciding the question. With respect to the query raised in the title of this paper, it can only be remarked for the present that valid evidence remains to be adduced that the ventricle exerts a suction action.

In conclusion I wish to extend my thanks to Doctor Wiggers in whose laboratory these experiments were carried out, and without prejudice to the present views of Doctor Katz, to thank him for his courteous and impersonal discussion of the whole matter.

SUMMARY

1. It is argued from theoretical considerations, that the experiments of Katz, from which the conclusion was drawn that "the ventricle not only can but does exert a sucking action to draw blood into its chamber," involves an experimental fallacy.

2. These theoretical arguments are further supported by experimental evidence, *showing that a purely passive system* can be utilised to reproduce the curves from which the active "suction action" was deduced.

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UTILIZATION OF VARIOUS CARBOHYDRATES BY THE DEPANCREATIZED ANIMAL

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Substitution for glucose of other carbohydrates which could be utilized without insulin has been attempted in the treatment of diabetes since the work of Külz (1874) and of Minkowski (1893) was done. Both of these authors noted that fructose behaves differently from the other sugars, both in normal and in diabetic subjects. This unusual utilization of fructose has been well established but has vanished when fructose was given continuously (Van Noorden and Naunyn). Recently, Roe and Schwartzman observed no difference between diabetic subjects and normal subjects in the tolerance for galactose, and suggested that galactose might be substituted for glucose in the treatment of diabetic subjects. Roe and Cahoon found that approximately half of the galactose they fed to depancreatized dogs was utilized, and that life without insulin could be prolonged by feeding galactose alone. Kosterlitz and Wedler found that galactose was excreted by depancreatized dogs completely as glucose or galactose. It was not possible to demonstrate on the partially insulinized animal that galactose is more available for oxidation than is glucose. Among diabetic patients, however, they found glycemia and glucuresis to be less with administration of galactose than with similar amounts of glucose. Campbell and Markowitz found no difference in the utilization of dextrose, levulose or inulin by the depancreatized dog. Joslin reported the case of a patient with diabetes whose urine remained sugar-free following assimilation of 100 grams of inulin, but glycosuria and hyperglycemia developed when a similar amount of potato starch was ingested.

In our experiments the depancreatized animals have been maintained in good general condition by the injection of sufficient insulin to prevent the fasting animal from excreting a significant amount of glucose. Our basal diet which contained about 60 grams of carbohydrate, gave rise to the excretion of a small amount of glucose. Under these conditions the feeding of additional carbohydrate could be studied by analysis of the feces to check absorption and by analysis of the urine to determine the total excretion of carbohydrate. In this way we have compared the utilization of glucose, fructose, galactose, sucrose, corn starch, and inulin by adding 50

grams of each to the basal diet during different periods. Since the amount of glucose appearing in the urine when glucose was fed was slightly greater than the amount of glucose added to the basal diet, it was obvious that none of the added glucose was utilized. When other carbohydrates were substituted and the excretion of carbohydrate was the same as with glucose, it also appeared that none of this carbohydrate was utilized unless it simply replaced glucose. When less carbohydrate appeared in the urine and absorption from the intestine was complete, the retained carbohydrate may have been oxidized or stored in the body.

METHODS. Under ether anesthesia, dogs weighing from 5 to 8 kgm. were depancreatized. Following operation the animals were given a mixed diet in excess of their requirements, and maintained by injection of 5 to 20 units of insulin twice daily, the amount of insulin varying with the amount of food the animal consumed. When the animals had regained their original weight and were in good condition they were given a diet consisting of 75 grams fresh meat, 25 grams fresh pancreas, 25 grams cracker meal and 100 cc. of milk twice daily. Ten units of insulin were injected at the time of each feeding. This treatment allowed the animals a slight gain in weight, and the urinary excretion of sugar varied from a trace to 25 grams daily. All of the animals in this experiment excreted more than 100 grams of glucose in twenty-four hours when this diet was given without insulin. When the animals were accustomed to the diet, the carbohydrate to be investigated was added to the basic diet; 25 grams of carbohydrate were added to each feeding twice daily. This regimen was continued for five days with each substance investigated, and during the next two days glucose was substituted for the carbohydrate and the administration of insulin was increased to 10 units twice daily. This procedure maintained the animals with little change in weight or condition.

The urine was collected every twenty-four hours by catheterization; this was then added to the urine collected from the metabolism cages during the interval. Strong hydrochloric acid was used as a preservative of the urine collected from the metabolism cages. The feces were collected daily, but most determinations were made on samples of mixtures of five days' collections.

Determinations of sugar were made by the Shaffer-Hartman method, and the figures obtained were calculated as glucose. No correction was made for urine that contained fructose or galactose. Such corrections would increase the value obtained for the carbohydrate excreted, but would not be greater than the observed daily variation, and would not alter our interpretations of results. The hydrolyzable carbohydrate of the feces was determined by hydrolysis in dilute solution with tenth normal hydrochloric acid at 100°C. for one hour, with subsequent determinations of sugar by the Shaffer-Hartman method.

RESULTS. The results reported here were all obtained by the addition, to the basal diet, each day, of 50 grams of the carbohydrates investigated, and the subcutaneous injection of 6 units of insulin into the depancreatized

TABLE 1

Excretion of sugar by depancreatized dog following addition of 50 grams carbohydrate to the basal diet

DAYS	GLUCOSE	GALACTOSE	FRUCTOSE	SUCROSE	STARCH*	INULIN*
	grams	*grams*	*grams*	*grams*	*grams*	*grams*
1	45.1	41.6	16.8	46.6	25.7	5.6
2	46.5	55.4	16.6	44.7	35.8	4.4
3	52.7	63.2	15.8	34.6	38.4	6.3
4	60.8	56.6	20.5	42.6	28.0	18.7
5	68.2	45.5	19.5	63.9	32.0	7.1
Average...	54.7	52.5	17.8	46.5	32.0	8.4

Dog 1 weighed 4.8 kgm. to 4.4 kgm. Basal diet 150 grams meat, 50 grams pancreas, 50 grams cracker meal, 200 cc. milk, 6 units insulin daily.

* Feces contained 18.6 grams hydrolyzable sugar daily during feeding with starch, 45.1 grams during feeding with inulin, and 1.0 to 1.8 grams during feeding with sugar.

TABLE 2

Excretion of sugar by depancreatized dog following addition of 50 grams carbohydrate to the basal diet

DAYS	GLUCOSE	GALACTOSE	FRUCTOSE 1	FRUCTOSE 2	FRUCTOSE 3	SUCROSE	STARCH*	INULIN*
	grams	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
1	51.7	62.0	14.4	51.8	59.8	50.8	46.1	19.0
2	62.5	63.8	23.5	55.4	65.5	49.6	52.1	19.2
3	73.7	78.6	28.3	53.8	66.3	53.1	66.5	20.3
4	80.1	60.4	28.8	57.1	62.7	52.1	43.4	43.3
5	87.7	77.8	31.0	62.9	63.6	53.0	43.0	19.3
Average...	71.1	68.5	25.2	56.2	63.6	51.7	50.2	24.2

Dog 2 weighed 5.2 to 4.5 kgm. Basal diet 150 grams meat, 50 grams pancreas, 50 grams cracker meal, 200 cc. milk, 6 units insulin daily. Fructose[2] given one month after fructose.[1] Fructose[3] given after three weeks' continuous administration of fructose.

* Feces contained 5.8 grams of hydrolyzable sugars daily during the feeding of starch; 37.4 grams during the feeding of inulin; 0.7 to 1.0 gram during periods when sugar was fed.

dogs. Although the utmost care was exercised to keep the daily routine of the animals constant, the results reported in tables 1 and 2 indicate considerable daily variation in the amount of sugar excreted under similar treatment. Our preliminary observations, however, convinced us that

this regimen was the most satisfactory. If insulin was not given, the amount of sugar excreted each day depended on the previous treatment of the animal, and the amount of glucose liberated from the animal's own stores often overshadowed the amount of carbohydrate in the diet. In experiments in which 10, 15 or 20 units of insulin were given daily, the variation of sugar excreted in the urine was more marked; in similar experiments, the total amount of sugar might be from 1 to 40 grams daily. This variation appeared to depend largely on the relation in time between administration of the insulin and the taking of the diet, less sugar being excreted when the animal ate all its food rapidly than when part of the food was eaten an hour or two after the insulin was administered.

There were variations in the appetite of all our animals, which we were unable to control: Sometimes they ate all of their food immediately, at other times they took only small portions at irregular intervals throughout the day, or they did not eat until the end of the day. The variations in the amount of glucose excreted were not so large as to prevent comparisons of periods of several days, but might be misleading if only one-day periods were used. The variations produced in the excretion of galactose and fructose when these substances were fed were much greater, larger amounts of these substances being excreted when the food was all taken at one time. Since we were not concerned with the conversion of galactose and fructose to glucose, but with the total sugar excretion with comparable diets, the amounts of these sugars in the urine were included as total urinary sugar.

Animals which received the basal diet without additional carbohydrate excreted from 2 to 25 grams of glucose when 6 units of insulin were given each day. Individual animals did not show such a wide variation, but maintained a daily excretion which varied by only a few grams of glucose at the most. The excretion of nitrogen was constantly about 6 grams, and there was a definite tendency to increase in excretion of nitrogen by the animals which excreted the larger amounts of sugar. When 50 grams of additional carbohydrate were absorbed, and excretion of glucose approximated 50 grams, the excretion of nitrogen remained unchanged. When the excretion of glucose under these conditions approached 80 grams the excretion of nitrogen rose approximately to 7 grams daily. The increased excretion of sugar could not be accounted for by the increased catabolism of protein, and calculations based on the excretion of nitrogen seemed of no positive value in this study.

The addition of 50 grams of glucose to the basal diet was followed by the excretion of approximately 50 grams of additional glucose in the urine. Animals which almost completely utilized the carbohydrate of the basal diet evidenced a tendency toward utilization of a small amount of the added glucose. Animals that excreted more glucose on the basal diet did not give evidence of this tendency. Analysis of the feces revealed that all of

the glucose had been absorbed, and that less than 1 gram of the hydrolyzable carbohydrates of the basic diet remained in the feces.

If 50 grams of raw corn starch were added to the basal diet, glycosuria was produced comparable to that obtained when glucose was fed. Less glucose appeared in the urine, but when the feces were examined it was found that from 5 to 20 grams of hydrolyzable carbohydrate had passed through the intestinal tract each day. The larger amounts of unabsorbed carbohydrate were found in the feces of animals which had a tendency to diarrhea. The amount of carbohydrate in the feces when added to the amount of glucose recovered in the urine, was approximately 50 grams or more, in those animals which excreted a larger amount of glucose when on the basal diet.

The addition of 50 grams of galactose to the basal diet was followed by increased excretion of approximately 50 grams of reducing substances in the urine. All of the galactose was absorbed from the gastro-intestinal tract, and some galactose appeared unchanged in the urine. Under these conditions determinations of galactose were made in a few experiments, which revealed that about a fifth of the galactose administered appeared unchanged in the urine, and that four-fifths appeared to have been converted to glucose and excreted as glucose in the urine. We found no evidence of the glucose sparing action of galactose.

All of the galactose added to the diet was apparently excreted in part as galactose, but for the most part as glucose. Continued galactose feeding, with administration of 6 units of insulin each day for four weeks, produced similar results. Contrary to what one might expect from the observations of Roe and Cahoon, our animals lost weight when fed galactose and progressively appeared to be in poorer condition, which did not improve until larger doses of insulin were administered.

If 50 grams of fructose were added to the basal diet, there was definite retention of carbohydrate. Only a small amount of fructose and only from 15 to 30 grams of glucose appeared in the urine. Analysis of the feces revealed that less than 1 gram of hydrolyzable carbohydrate had remained unabsorbed each day. All animals showed, also, a slight but definite decrease in the excretion of nitrogen when fructose was fed, compared to that when glucose was given. After the first five days that fructose was fed, the excretion of glucose increased, and within ten days the excretion of glucose was similar to that when glucose was administered. There was also a slight increase in the excretion of nitrogen. Four animals apparently thus utilized fructose when fructose was first administered. Subsequent administration of fructose, after periods of five days of administration of fructose, revealed greater excretion of glucose. It appeared to take about a month, without fructose, to restore the animal to the original state for utilization of fructose. Single administrations of fructose at intervals longer than one day were not attempted.

The addition of 50 grams of sucrose to the basal diet was followed by the excretion of slightly less than the equivalent amount of glucose in the urine. All of the sucrose was absorbed from the gastro-intestinal tract, and none of the unchanged sucrose was found in the urine. From the results obtained, it would appear that the fructose portion of the sucrose molecule was partly retained as it was when fructose was fed.

The addition of 50 grams of inulin[1] to the basic diet was followed in most cases by only a small increase in the excretion of glucose. Fructose was not found in the urine. Feeding of inulin seemed at times to produce diarrhea, and a large amount of the substance given appeared in the stools as hydrolyzable carbohydrate. Usually about 40 grams of hydrolyzable carbohydrate were recovered in the feces after feeding 50 grams of inulin. Considerable variation, however, was observed; some animals excreted almost 50 grams daily whereas others excreted about 20 grams of inulin in the feces. The excretion of sugar in the urine was definitely higher by animals which gave more evidence of hydrolysis of inulin and absorption of fructose. The excretion of sugar, however, did not appear to be as great as would have been expected if starch had been fed and glucose absorbed. Normal animals appeared to hydrolyze inulin much better than depancreatized dogs. Fifty grams of inulin added to the same basal diet of normal dogs revealed that from 5 to 15 grams of hydrolyzable carbohydrate remained in the feces each day. It would appear probable that diabetic patients might absorb more fructose from inulin than the depancreatized dog absorbs. It would also seem probable that small amounts of fructose could be retained without the administration of additional insulin.

SUMMARY

The addition of 50 grams of carbohydrate to the basal diet of depancreatized dogs which received 6 units of insulin daily is followed by increased excretion of glucose in the urine. The amount of carbohydrate in the urine is similar if glucose or galactose is fed, although some of the galactose appears unchanged in the urine. Corn starch acts in a manner similar to glucose if consideration is made for the amount of unhydrolyzed starch in the feces. Fructose or sucrose (probably the fructose portion of the sucrose molecule) appears to be partially utilized under these conditions for several days. This apparent utilization is soon lost, however, and the total amount of sugar added to the diet appears in the urine as glucose. Inulin is only slightly hydrolyzed in the intestine of the depancreatized dog, but such portions as are hydrolyzed produce effects similar to equivalent amounts of fructose.

[1] The inulin used in this experiment was obtained through the courtesy of Dr. H. S. Paine, Chemist in Charge, Carbohydrate Division, Bureau of Chemistry and Soils of the United States Department of Agriculture, Washington, D. C.

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ON THE FUNCTION OF THE ADRENAL CORTEX—GENERAL, CARBOHYDRATE AND CIRCULATORY THEORIES

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Considerable controversy has arisen within the past year regarding the function of the adrenal cortex. Three noteworthy theories have been advanced. The first in order of appearance, presented by this laboratory nearly two years ago (Britton and Silvette, 1932b), postulates that the adrenal cortex prepotently regulates carbohydrate metabolism; the second theory (Hartman et al., 1932) considers that the cortex produces a general tissue hormone; while a third which has recently appeared (Swingle et al., 1933) relates cortical function to regulation of the circulating blood volume.[1]

It has been apparent for many years that the adrenal cortex has an important relationship to circulatory activities in the body. Three-quarters of a century ago Addison (1849, 1855) observed that the adrenal glands were "directly or indirectly concerned with sanguification." Nearly twenty years ago Elliott (1914) showed that an apparent paralysis of the vasomotor mechanism which followed adrenalectomy was possibly responsible for death of the animal. In one section of a review a few years ago, attention was drawn to more than a score of important observations on the subject (Britton, 1930). In further articles from this laboratory (Britton and Silvette, 1931a; Corey and Britton, 1932) the effects of adrenalectomy and of cortico-adrenal extract on the circulation have been recorded and evaluated. Antedating by two years the recent report of Swingle et al. there also appeared a comprehensive experimental study by Wyman and tum Suden (1930) on "The blood volume in suprarenal insufficiency, anaphylactic shock and histamine shock."

In their article on blood-volume regulation and the relation of the adrenals to shock, Swingle and his associates (1933) give no attention to the foregoing and many other pertinent contributions. Indeed, regarding other investigations on the subject they affirm that "none have materially

[1] Observations now put forward by some of Swingle's colleagues (Harrop et al., 1933) indicate that the adrenal cortex may be chiefly concerned in sodium-chloride regulation in the body.

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advanced the problem of cortical function." In a dozen "bald statements," so designated by themselves, they present what appear to be their own conclusions—and these are apparently justification for a later declaration that "there can be no question but that the adrenal cortical hormone is specific in the treatment of surgical and traumatic shock" (see Cattell, 1933).

Brief preliminary replies to the Princeton article show clearly that the subject is not at all as settled as implied (Freeman, 1933; Britton and Silvette, 1933). From observations over the past few years we are led to believe that changes in blood pressure and in the amount of circulating blood are indirect and late manifestations of adrenal removal, and illustrative only of the general effects of adrenalectomy on the animal. The cessation of gastro-intestinal activity and the suppression of urinary secretion which occur in adrenal insufficiency are further examples in this respect. Probably the circulatory changes are the result of more fundamental disturbances occurring in the vital tissues or organs of the adrenalless animal. These considerations and the publicity given to the Swingle theory (see Cattell, 1933) have prompted an extension of our earlier experiments on circulatory changes in adrenal insufficiency, as well as a more critical analysis of our carbohydrate theory of cortico-adrenal function.

We have sought an understanding of the more fundamental chemical changes and conditions in adrenal insufficiency which, because of their crucial nature, are antecedent to or take precedence over any altered permeability relationships in the body. Three years ago one of us, after an extended analysis of the data at hand, concluded that the adrenal cortex is chiefly concerned with the storage and utilization of carbohydrates (Britton, 1930). Experimental evidence which we presented nearly two years ago gave support to this theory (Britton and Silvette, 1932 a, b). It may now be said that breakdown to the extent of utter collapse of the normal metabolism of carbohydrates in the organism appears to us still, in the light of further evidence herein reported, to constitute the first critical contingency in adrenal insufficiency. Investigations which we have carried out during the past year on urinary secretion, blood volume and water balance do not lead us, on the other hand, to attribute any outstanding significance to the body fluid changes which have been observed in these experiments.

In the preliminary article of Swingle and his collaborators (1933), which appeared at the time our first full report on water balance and urinary secretion in adrenalectomized animals was ready for publication, it was proposed that "the function of the adrenal cortical hormone is the regulation and maintenance of a normal circulating volume of fluid within the vascular system." It was further considered that "all the manifestations, symptoms and peculiarities which have been described as occurring

in adrenalectomized animals are merely results of a progressively failing circulation, due to decreasing volume of circulating fluid." Our experiments described below are pertinent to these contentions; the later investigations were designed to test more rigorously our own carbohydrate theory.

METHODS. The water content of the tissues was determined by weighing the fresh material in covered aluminum pans, and drying to constant weight in an electric oven at 108°C. Blood-cell volume determinations

TABLE 1

Percentage of water in blood, liver and muscle of normal and adrenalectomized cats

CAT NUMBER	WEIGHT	CONDITION	WHOLE BLOOD WATER	LIVER WATER	MUSCLE WATER
		Unoperated			
	kilos		*per cent*	*per cent*	*per cent*
11	2.14	Normal	86.8	66.6	74.2
12	1.98	Normal	82.8	69.3	74.7
13	1.80	Normal	85.2	71.7	74.8
14	1.30	Normal	85.6	71.4	75.8
15	1.80	Normal	84.8	70.6	75.2
16	1.44	Normal	85.0	67.2	73.3
27	2.55	Normal	85.8	71.2	74.7
29	2.41	Normal	83.2	67.3	74.2
		Adrenalectomized			
17	1.47	Prostrated	80.7	74.3	75.9
18	2.36	Weak	81.1	73.0	74.6
19	1.11	Prostrate	81.0	73.4	77.5
20	3.56	Very weak	77.1	74.1	77.5
21	2.24	Prostrated	82.5	73.3	76.4
22	2.18	Prostrated	80.2	74.2	74.5
23	2.38	Prostrated	78.7	73.9	72.6
24	2.98	Prostrated	82.1	71.4	81.2
25	2.01	Prostrated	82.4	74.1	75.0
26	1.62	Prostrated	79.9	69.8	74.1
30	1.86	In convulsions	77.3	72.0	73.6

were made after the method of Van Allen (1925). Blood volume was determined with slight modifications according to the method of Cartland and Koch (1928). One cubic centimeter of 2 per cent brilliant vital red was injected rapidly into the heart and exactly three minutes later 1 cc. of the dyed blood was removed, the needle having been *in situ* throughout the interval. While inherent sources of error were appreciated, the method was considered the most suitable for the experiments undertaken, and numerous control tests were found to give fairly well duplicated readings.

Blood-glucose and tissue-glycogen determinations were made according to methods previously described (Silvette and Britton, 1932). Other experimental procedures are indicated later.

RESULTS. *Blood volume and tissue fluids.* The data given in tables 1 and 4 reveal clearly that a *shift* in fluids occurs in the tissues of the adrenalectomized animal, the movement being away from the blood toward the liver and muscle tissues. In an earlier report we have shown that water in the tissues other than the liver and muscle is practically unaffected (Silvette and Britton, 1933).

TABLE 2

Effects of fluid injections on the water percentage of blood and tissues of adrenalectomized cats

(Injections given when early symptoms of insufficiency appeared)

CAT NUMBER	SURVIVAL AFTER OPERATION	DETERMINATIONS BEFORE TREATMENT		AMOUNT OF SALINE INJECTED*		TIME BETWEEN INITIAL AND FINAL SAMPLES	DETERMINATIONS AFTER TREATMENT			
		Whole blood, water	Blood cell volume	Intracardial	Intraperitoneal		Whole blood, water	Blood cell volume	Liver water	Muscle water
	days	*per cent*	*per cent*	*cc.*	*cc.*	*hours*	*per cent*	*per cent*	*per cent*	*per cent*
153	8	83.6	49		450	11	85.0	39	73.9	77.0
154	7	82.4	56		600	8.5	84.4	35	78.1	80.2
155	9	85.2	40		300	10	85.3	36	75.0	79.4
159	5	85.5	40	40	100	5		32	71.3	76.4
168	3	79.7	53	25	75	1	87.9	32	77.6	76.2
171	1	84.9	43	20		1	85.9		75.3	78.3
173	2	83.6	48	24	75	2	85.4	28	72.3	76.0
205	3	80.3	51	30		1	86.2	22		
206	2	80.1	53		120	3	82.4	52	75.4	76.8
207	2	83.1	44		120	3.2	86.1	44	74.0	76.5

* All intraperitoneal injections, isotonic saline. Intracardial injections: Nos. 168, 171, 173, 15 per cent gum-saline; no. 159, isotonic saline; no. 205, 7 per cent gum-saline.

The blood volume, water percentage and hematocrit readings considered together give an intimate picture of changes which take place in the circulating blood. Two to four days after operation the percentage of water in the blood is only slightly reduced, and the blood-cell readings slightly increased, compared to the normal. When severe adrenal insufficiency has developed these changes are accentuated, and the total blood volume is found to be reduced. In 10 normal cases (cats) there was an average volume of 6.3 per cent, and in 13 adrenalectomized cases an average of 5.2 per cent, representing a difference of about 20 per cent. The readings were as follows: Normal cats: blood volumes per cent—6.4, 6.0, 6.3, 6.0, 6.1, 6.4, 6.9, 7.0, 6.4, 5.9. Adrenalectomized cats (showing symptoms

of insufficiency): blood volumes—5.7, 5.6, 4.8, 5.7, 5.7, 5.9, 3.7, 4.5, 6.1, 4.2, 4.3, 5.4, 5.7. Decreases in volume of 30 per cent below the normal average were observed in a few instances; nevertheless, in other cases in which adrenalectomized animals were known to be dying with severe symptoms of insufficiency, the blood volume was found to be within normal limits.

Observations which were made on adrenalectomized animals injected with saline or gum-saline solutions, after symptoms of insufficiency had developed, indicated that the blood volume may be fully restored, but apparently without affecting the condition of the animal in any way (tables 2 and 3). Life could not be prolonged in such cases; indeed, the usual progressive decline towards death invariably occurred. The blood volume

TABLE 3

Effect of fluid injections on the blood volume of cats showing symptoms of adrenal insufficiency

CAT NUMBER	CONDITION BEFORE INJECTION	BLOOD VOLUME BEFORE INJECTION	AMOUNT OF SALINE INJECTED*		TIME BETWEEN INITIAL AND FINAL SAMPLES	CONDITION AFTER INJECTION	BLOOD VOLUME AFTER INJECTION
			Intracardial	Intraperitoneal			
		per cent	*cc.*	*cc.*	*hours*		*per cent*
170	Weak	5.7	25	75	1.5	Convulsions	6.3
172	Weak	5.6		150	3.0	Very weak	7.7
201	Weak	5.7		120	5.5	Very weak	5.9
205	Weak	4.5	30		1.3	Prostrated	6.0
206	Sl. weak	4.2		120	3.0	Prostrated	5.8
207	Sl. weak	4.3		120	3.0	Prostrated	5.1
211	Sl. weak	5.4		120	3.8	Weak	6.1
212	Weak	5.7	30	120	4.3	Very weak	6.7

* All intraperitoneal injections, isotonic saline. Intracardial injections: nos. 170 and 212, 7 per cent gum-saline; no. 205, 15 per cent gum-saline.

was in some instances observed to be elevated even above the normal when the animal was in the later stages of insufficiency. It was apparent that adrenalectomized animals possess the ability to *draw* fluid into the blood vessels, and also to *retain* intravascularly injected solutions. There were also observed in these experiments notable accessions of fluid by the liver and muscle following the injections, emphasizing again the hydrophilic condition of the tissues in the adrenalectomized animal. Comparison of the foregoing conditions which are observed in normal and adrenalectomized animals may be made by reference to table 4.

Tests on blood sugar and glycogen reserves. A series of adrenalectomized rats which were showing symptoms of insufficiency has been examined. The rapidity with which the symptoms eventually develop and death

ensues in (about 60 per cent of) adrenalectomized rats makes it rather difficult, it may be said, to secure a large number of animals in the desired stage of insufficiency. Some animals often continue to take food and may appear almost normal up to the point of death. Only in two instances had severe symptoms set in at the time the analyses were made. In a major particular—that of the liver glycogen level—the results are consonant with our earlier work. The hepatic glycogen reserves were found to be markedly reduced at the time of development of the earliest symptoms of adrenal insufficiency in rats (table 5). In 6 cases there was an average of 0.17 per cent compared to an average of 1.05 per cent in control cases. The blood glucose is not, however, significantly reduced below the levels observed in fasting animals, and the muscle glycogen is apparently unaffected—results which will be considered later.

TABLE 4

Observations on blood and tissues of normal and adrenalectomized cats under various conditions

(Averages of 8 or more experiments)

CONDITION OF ANIMAL	WATER			BLOOD CELLS	BLOOD VOLUME	
	Liver	Muscle	Whole blood		Average	Range
	per cent	*per cent*	*per cent*	*vol. per cent*	*per cent*	*per cent*
Normal	69 4	74 5	84 9	42	6 3	5.9–7.0
Adrenalectomized, slight symptoms			82 8	48		
Adrenalectomized, severe symptoms	73.5	75.7	80 3	53	5.2	4 2–6 1
Adrenalectomized, after fluid injection	74 8	77 4	85 4	36	6 2	5.1–7 7

In adrenalectomized guinea pigs which were showing symptoms of insufficiency, both the liver glycogen and blood glucose values were profoundly reduced below the normal (table 5). The readings are in agreement with the carbohydrate changes previously observed in cats and quoted in brief herewith.

Similar profound changes were noted in adrenalectomized marmots or groundhogs (*Arctomys monax*). The blood sugar and glycogen reserves were reduced to levels which are known to be incompatible with life—the liver glycogen in a few instances being reduced to the disappearing point. The average liver glycogen in 6 adrenalectomized animals was 0.19 per cent compared to 2.4 per cent in fasting normal controls, and the average blood sugar 44 mgm. in contrast to 162 mgm. per 100 cc. in the control animals. Muscle glycogen was also much lower in adrenalectomized groundhogs (table 5).

TABLE 5

Blood sugar and liver and muscle glycogen levels in normal and adrenalectomized rats, guinea pigs, cats and marmots

	ANIMAL NUMBER	SURVIVAL	CONDITION WHEN KILLED	LIVER GLYCOGEN	MUSCLE GLYCOGEN	BLOOD SUGAR
		days		*per cent*	*per cent*	*mgm. per cent*
Rats:						
Unoperated, 6 cases*			Normal	1.05	0.466	113
Adrenalectomized	1	6	Weak	0.236	0.347	71
	6	6	Prostrated	0.166	0.216	73
	7	6	Sl. weak	0.194	0.316	78
	8	13	Weak	0.138	0.248	
	9	14	Very weak	0.223	0.442	100
	10	11	Prostrated	0.071	0.201	66
Guinea pigs:						
Unoperated	1		Normal	2.15	0.363	98
	2		Normal	1.72	0.381	102
	3		Normal	1.90	0.464	89
	4		Normal	2.10	0.630	88
Adrenalectomized	5	1	Prostrated	0.245	0.286	50
	6	1	Convulsions	0.241	0.475	43
	7	2	Convulsions	0.210	0.415	41
	8	1	Prostrated	0.109	0.238	77
	9	1	Prostrated	0.084	0.289	61
Cats:†						
Unoperated, 11 cases			Normal	1.22	0.426	92
Adrenalectomized, 10 cases		2–8	Symptoms	0.067	0.208	44
Marmots:						
Unoperated	1		Normal	2.36	0.96	185
	2		Normal	2.77	0.57	160
	3		Normal	2.24	0.83	132
	6		Normal	2.56	0.54	144
	8		Normal	2.21	0.67	186
	21		Normal	2.24	0.77	132
	22		Normal	4.33	0.89	144
	23		Normal	2.32	0.37	170
	24		Normal	1.63	0.77	189
	30		Normal	1.32	0.49	178
Adrenalectomized	7	3	Prostrated	0.070	0.34	49
	9	4	Comatose	0.079	0.17	49
	10	2	Convulsions	0.238	0.51	49
	11	8	Prostrated	0.350	0.53	29
	12	19	Convulsions	0.161	0.47	44
	25	2	Convulsions	0.283	0.43	45

* From table 4, Britton and Silvette, 1932, This Journal, **100**, 693.

† From table 6, Britton and Silvette, 1932, This Journal, **100**, 701.

Emotional responses and adrenalin tests. The presence or availability of hepatic glycogen reserves in the adrenalectomized animal has been

TABLE 6

Effects of adrenalin injection on the blood sugar of normal and adrenalectomized cats*

SERIES	CAT NUMBER	CONDITION	DAYS BETWEEN OPERATION AND EXPERIMENT	BLOOD SUGAR				
				Initial	After injection			
					30 min.	60 min.	120 min.	180 min.
				mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
A_1	160	Normal		114	174	180	154	138
	161	Normal		80	192	163	121	82
	162	Normal		61	177	141		58
	164	Normal		75	161	127		103
A_2	242	†Adrex	2	102	92	106	90	70
	249	Adrex	3	69	56	61	57	39
	250	Adrex	3	86	104	104	97	96
	250	Adrex	4	73	70	70	65	64
	256	Adrex	1	56	59	60	62	65
	256	Adrex	2	53	64	64	64	63
	258	Adrex	1	82	89	86	78	76
	258	Adrex	2	74	87	84	78	76
	251	Adrex	5	72	65	64	63	63
B_1	165	Normal		74	230	236		84
	166	Normal		86	230	204		79
	167	Normal		80	144	183		106
	168	Normal		96		185		
	169	Normal		75		206		
B_2	263	Adrex	3	99	206	196		108
	265	Adrex	3	76	148	161		101
	266	Adrex	7	48	53	66		55
	256	Adrex	4	53		57		
	258	Adrex	4	67		139		
C_1	170	Normal		81	333	425		308
C_2	261	Adrex	4	59	74	114		77

* Adrenalin HCl solution injected intraperitoneally, 10 cc. per kilo; in all cases: Groups A_1 and A_2, concentration 1 in 200,000; B_1 and B_2, 1 in 50,000; C_1 and C_2, 1 in 10,000.

† Adrex = Adrenalectomized.

tested by subjecting cats to profound emotional excitation by a barking dog. The cats were used at various periods after complete adrenalectomy, while apparently in perfect health; after initial fasting blood samples had

been taken, they were exposed (while in a wire cage) to an aggressive dog for 4 minutes. At the end of such excitation the final blood samples were drawn. The results were as follows:

Cat A, 3 days after operation, initial blood sugar 99 mgm., final (after exciting) 110 mgm. per 100 cc.; 4 days after operation, 94 mgm. initial to 95 mgm. per 100 cc. after exciting; at 5 days, 99 mgm. to 101 mgm. Cat B, at 4 days, 86 mgm. initial to 91 mgm. after exciting. Cat C, at 5 days, 86 mgm. initial to 87 mgm. after exciting.

The negligible changes noted in adrenalectomized cats are in striking contrast to the 50–100 per cent increases in blood sugar which have been

TABLE 7

*Blood sugar and liver and muscle glycogen levels in normal and adrenalectomized cats after sodium lactate injection**

CAT NUMBER	CONDITION WHEN SACRIFICED	BLOOD SUGAR		TERMINAL GLYCOGEN		DAYS BETWEEN OPERATION AND EXPERIMENT
		Initial	Final	Liver	Muscle	
		mgm. per cent	*mgm. per cent*	*per cent*	*per cent*	
101	Normal	116	124	2.61	0.727	
102	Normal	76	81	1.33	0.547	
103	Normal			1.72	0.894	
104	Normal	108	133	1.86	0.790	
105	Normal	107	139	2.59	0.650	
106	Normal	110	141	1.89	0.700	
107	Normal	82	127	2.57	0.421	
108	Normal	78	99	2.26	0.407	
255	Adrenalectomized	83	102	0.208	0.343	5
257	Adrenalectomized	97	136	0.341	0.492	4
254	Adrenalectomized	77	149	0.159	0.515	6
263	Adrenalectomized	94	103	0.136	0.377	3
265	Adrenalectomized	68	75	0.218	0.628	3
272	Adrenalectomized	70	80	0.308	0.564	3

* One intraperitoneal injection of 10 cc. per kilo of 8 per cent sodium *dl*-lactate at 0 hours, another at 2 hours; animal killed at end of 4th hour.

commonly observed on similarly exciting normal animals in this and other laboratories (see Britton, 1928).

In correlation are several series of experiments in which the effects of adrenalin solutions of various concentrations were tested on adrenalectomized animals, and comparison made at the same time with normal cases. The results are given in table 6. It is to be noted that tests were made at various time intervals after operation, and that in all cases the animals appeared to be in good health when injected. Particularly it will be observed that while the weakest solutions of adrenalin used brought about increases of over 100 per cent in blood sugar in normal cats (an average

of 83 to 176 mgm. per 100 cc.; see table 6, A_1; also figs. 1 and 2), such concentrations of adrenalin were practically without effect on adrenalectomized animals (A_2). In the latter operated group the average variation in 9 cases was from 74 mgm. (initial) to 76 mgm. (30 minutes after adrenalin injected). While strong doses of adrenalin brought about blood-sugar increases in the case of a few recently-operated cats, even very high and

TABLE 8

Effects of glucose injection on blood glucose and liver and muscle glycogen levels in normal and adrenalectomized cats

SERIES	CAT NUMBER	CONDITION	DAYS BETWEEN OPERATION AND EXPERIMENT	GLUCOSE INJECTED (10 PER CENT)	BLOOD SUGAR						TERMINAL GLYCOGEN*	
					Initial	After injection						
						30 min.	60 min.	120 min.	180 min.	240 min.	Liver	Muscle
			days	*cc./kilo*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*per cent*
A_1	110	Normal		10	88	159	175	156	114			
	111	Normal		10	76	160	168	159	109	102		
	112	Normal		10	82	154	167	149	109	108		
A_2	233	Adrex	8	10	82	290	290	247	200	96		
	234	Adrex	8	10	105	286	244	168	119	76		
	239	Adrex	3	10	106	233	239	242	141	115		
B_1	113	Normal		10	82		153				1.22	0.51
	114	Normal		10	112		170				2.54	0.46
	115	Normal		10	103		180				1.92	0.43
B_2	241	Adrex	4	10	52		179				0.53	0.40
	244	Adrex	4	10	90		225				0.42	0.21
	267	Adrex	3	10	100		209				0.42	0.64
C_1	116	Normal		30	80	288	312	299	231	194	3.47	0.61
	117	Normal		30	75	286	286	258	169	139	3.63	0.58
C_2	248	Adrex	2	30	98	354	284	270			0.23	0.52
	274	Adrex	4	30	78	245	240		206	169	0.59	0.46

* Animals sacrificed after taking last blood sample.

severely toxic concentrations failed in several instances to elevate the blood glucose of adrenalectomized animals (table 6).

Glycogen storage: lactate and glucose administration. The severe carbohydrate deficiencies in the adrenalectomized animal which became apparent in the foregoing experiments suggested that inquiry also be made into glycogen synthesis after adrenal removal. Sodium lactate injections were given in one series of animals at different intervals after operation, and

comparison made with controls which were similarly treated. The animals were taken from the same pen and tested in the morning after having been without food for approximately 12 hours. The adrenalectomized animals were apparently in good health when injected, although in several cases the intraperitoneal lactate injection possibly hastened the onset of in-

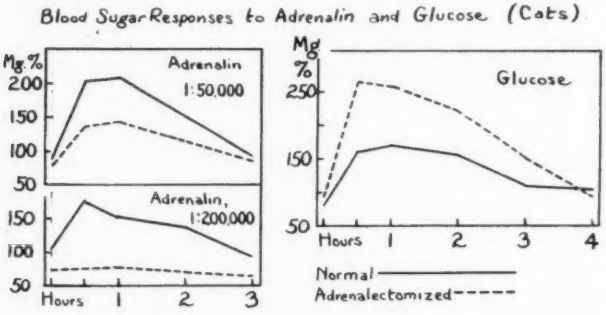

Fig. 1

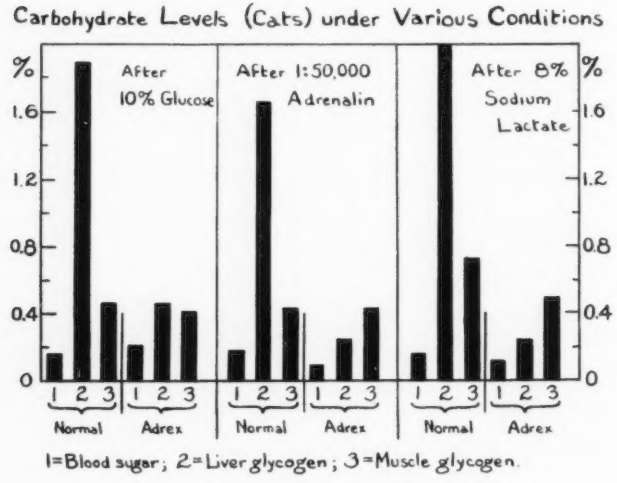

Fig. 2

sufficiency symptoms. The results are given in table 7. There is observed to be a definite inability on the part of the adrenalectomized animal to utilize lactate for glycogen formation, in contrast to normal animals. Hepatic glycogen in the latter, it may be noted, is 5 to 10 times higher than in those animals deprived of their adrenal tissues.

Under conditions similar to those noted above, the effects of glucose injections were observed on normal and on adrenalectomized animals. In several cases blood-sugar changes particularly were studied (table 8, A_1 and A_2); in others, attention was given to glycogen storage (B_1 and B_2). Similar amounts of glucose brought about a much greater augmentation of the blood-sugar level in adrenalectomized animals, it will be observed, in comparison to the unoperated controls. In correlation there was a notable failure on the part of the adrenalless animal to store glycogen, even under the influence of huge amounts of glucose (C_2; also figs. 1 and 2).

In a number of cases the effects of long-continued glucose injections in promoting glycogen storage have been tested on adrenalectomized animals. Ten cubic centimeters of a 5 per cent solution per kilo weight were given intraperitoneally twice daily. In all instances, however, the liver glycogen levels at the time the animals were sacrificed have been observed to be profoundly reduced. The following protocols are illustrative:

Cat 269, male; May 31, 1933, weight 2.9 kilos; both adrenals removed. June 1, 2, 3 and 4, two intraperitoneal injections of 29 cc. (10 cc. per kilo) 5 per cent glucose daily, morning and evening. June 5, a.m., animal killed. Liver glycogen, 0.09 per cent; muscle glycogen, 0.44 per cent; blood-sugar, 107 mgm. per cent.

Cat 271, male; May 31, 1933, weight 2.3 kilos; complete adrenalectomy. June 1, 2, 3 and 4, two intraperitoneal injections of 23 cc. (10 cc. per kilo) 5 per cent glucose daily, morning and evening. June 5, 9:00 a.m., given 23 cc. 5 per cent glucose intraperitoneally; 10:45 a.m., blood sugar 96 mgm. per cent; 11:35 a.m., animal killed. Liver glycogen, 0.11 per cent; muscle glycogen, 0.38 per cent; blood sugar 75 mgm. per cent.

Although large amounts of glucose were given throughout the postoperative period, storage of liver glycogen was apparently impossible. The blood-sugar levels were found normal, as will be noted, at the end of the experiments, in contrast to non-glucose-treated cases.

In three control cases in which normal cats were given the same amounts of glucose over the same period of time as the above adrenalectomized animals, the following data were obtained:

Cat 307, liver glycogen 3.36 per cent; muscle glycogen 0.814 per cent; blood sugar 90 mgm. per cent.

Cat 308, liver glycogen 2.47 per cent; muscle glycogen 0.845 per cent; blood sugar 86 mgm. per cent.

Cat 309, liver glycogen 2.84 per cent; muscle glycogen 0.627per cent; blood sugar 82 mgm. per cent.

Discussion. The adrenalectomized animal suffers from glucose and glycogen lack which becomes progressively more severe after the operation and parallels the development of symptoms. In 40 cases taken from our records, adrenalectomized cats with varying degrees of insufficiency—from very slight to severe—showed blood-glucose levels which averaged 46 mgm.

per cent, i.e., about one-half of the value which is observed in normal animals. Blood samples taken from animals before symptoms set in have, furthermore, often shown hypoglycemic levels. The liver has been found to be practically exhausted of glycogen—in 10 cases the levels averaged 95 per cent below normal—and the muscle glycogen percentage was reduced by more than 50 per cent. We have never observed more profoundly critical disturbances in any other condition involving carbohydrate metabolism—e.g., the cachexia following hepatectomy or pancreatectomy, or heavy insulin overdosage—which we have investigated.

Measures which ordinarily stimulate hyperglycemic reactions in normal animals fail to do so, it is now shown, in adrenalectomized animals. Adrenalin injections and emotional excitation affect scarcely at all the blood-sugar levels in adrenal insufficiency, even though the animals are apparently in good condition. Carbohydrate mobilization is impossible—glycogen reserves are not available in the liver. Furthermore, glycogen synthesis following glucose or sodium lactate injection does not occur to any significant degree in the adrenalectomized animal.

These observations lead to the conclusion that lack of the cortico-adrenal hormone brings about in a gradually progressive manner a profound disorganization of carbohydrate metabolism. The general decomposition of the organism which follows adrenalectomy is apparently explicable on the basis of carbohydrate deficiencies of a fundamental character. That the general condition of the animal brings about the carbohydrate disturbance is not tenable.

Animals dying of adrenal insufficiency show convulsive seizures some hours before death occurs which are identical in type with those observed in insulin hypoglycemia. The convulsions appear indubitably related to the reduction in circulating glucose; they may properly be considered as the immediate (general) cause of death. By contrast, we have not observed such convulsions in animals in which the circulating blood volume has been markedly reduced by various operative procedures. In numerous experiments on shock produced by blood withdrawal and other measures, convulsive symptoms—which are known to be characteristic of adrenal insufficiency (as well as hypoglycemia), and which result in death—do not occur. Without further analysis of the statement of Swingle and his collaborators (1933) that "all the symptoms . . . . are merely results of a progressively failing circulation," it is sufficient to point out that lack of consonance with fact in regard to the most obvious symptom of adrenal insufficiency puts the circulatory theory at least in jeopardy.

An early contention by Swingle (1927) that carbohydrate deficiency in the adrenalectomized animal is of considerable if not of primary importance is interesting. It has also been shown by Cori and Cori (1927) that adrenalectomized animals (rats and mice) which have been fasted for short periods show an almost complete disappearance of liver glycogen and a

marked reduction in blood sugar. In adrenalectomized mice, hypoglycemic convulsions sometimes supervened, and these were promptly abolished by the administration of glucose.

In a large series of recent experiments we have never observed any amelioration of the general condition of adrenal insufficiency following the administration of large amounts of saline solution by intraperitoneal or intracardial injection. The circulatory volume is nevertheless restored readily to normal in these cases.

Glucose injections may prolong two- or three-fold the survival period of adrenalectomized animals, however, and abolish the severe symptoms when these are allowed to develop (see Britton, 1930; also unpublished results). Yet carbohydrate deficiencies do not explain the whole problem of adrenal insufficiency, and the apparent ineffectiveness of simple glucose solutions in maintaining indefinitely the life of an adrenalectomized animal is admittedly inexplicable at this time. It may be recalled that glucose is only temporarily effective in restoring animals from the early coma which follows hepatectomy. We have observed too that in a number of cases glucose does not restore severely insulinized animals. It is to be emphasized that we have been concerned simply with *the first crucial signs of failure* in the adrenalectomized animal. That there occurs a disturbance primarily and fundamentally in glycotaxis in the body is suggested as most fitting the evidence at hand. Adrenal insufficiency may perhaps be considered as a sort of glycoprivic intoxication.

We have observed that cortico-adrenal function is very intimately related to carbohydrate metabolism in rats, rabbits, cats, guinea pigs, groundhogs and opossums. The apparent fact that the liver glycogen alone (and not the blood sugar) is notably reduced in adrenalectomized rats is probably due to the inherent difficulty of completely removing all the cortico-adrenal tissue in these animals. It seems doubtful whether adrenalectomized rats die from true adrenal insufficiency. We have not carried out tests on dogs because of the large amounts of extract demanded and the consequent great expense involved.

It has been previously shown (Britton and Silvette, 1932b) that carbohydrate derangements such as those described above which develop in adrenalectomized animals are rapidly alleviated by adequate dosage with cortico-adrenal extract. In young cats, particularly, the effect of the extract on the hypoglycemic, deglycogenized condition which follows adrenal removal is well observed. The restorative effects readily occur, indeed, when the extract is administered by mouth.

Swingle and his associates (1933) state that in cases of adrenal insufficiency blood dilution "never occurs, unless the hormone is injected." In our experiments we have observed that blood dilution *does* occur and restoration of the normal circulatory volume in adrenalectomized animals may

readily be brought about by injections of saline solution. The symptoms of insufficiency are not at all relieved, however, nor is life even slightly prolonged—in sharp contrast to the restorative effects (although temporary) of glucose administration.

It is interesting to note that one of Swingle's colleagues (Rowntree, 1925) found that in 10 cases of adrenal insufficiency in man (even in the severe coma of Addison's disease) the blood volume is *not* diminished, but is *per contra* normally well maintained. In their experiments on adrenalectomized dogs Kendall and his collaborators (1933) observed, moreover, that while the blood volume was low at the time of death, this was only one of the many changes which developed, and did not appear to be the primary cause of death.

It has been repeatedly shown, in contrast to the above, that the blood sugar is very commonly low, and profound hypoglycemia may be present, in Addisonian affections. Several years ago Porges (1910) noted that the symptoms in Addison's disease were similar to those of hypoglycemia, with weakness and fatigue as early signs and convulsions and stupor as later phases of the condition. In his three cases Porges observed that severe hypoglycemic levels were present. Rowntree (1925) found the blood glucose below normal in nine cases, and in two cases it was critically reduced to 45 mgm. per cent. Levy Simpson (1932) found the blood sugar almost constantly subnormal in six cases which he reported. The value of glucose administration in the coma of Addison's disease is well known.

Our observations do not lead us to support the theory of Hartman (1932) that the adrenal cortex provides a general tissue hormone to the body. With Hartman one must agree, to be sure, that the activities of the adrenal cortex have extraordinarily wide ramifications. Lack of the hormone is accompanied by profound disturbance of the digestive, circulatory, neuromuscular, and renal and genital systems. These extensive changes appeal to us, however, as representing systemic or merely general, apparently secondary effects, brought about by a more fundamental disorganization in body chemistry due to absence of the cortical hormone.

Two contrasting facts stand out clearly from our studies of the past few years: *The adrenalectomized animal with symptoms of insufficiency shows an almost complete and thus very critical depletion of its reserve and circulating carbohydrate materials. It does not under similar circumstances show a lack of water in the body.*

The increased amounts of water which are found in the hepatic and muscular tissues of the adrenalectomized animal balance the loss of fluid from the blood stream. We derive an impression from our data that water is more necessary for the maintenance of normal conditions in the liver and muscle than for any demands of the circulation. It is to be remembered furthermore that one of the earliest effects of cortico-adrenal

extract which we have observed is the *elimination* of fluid from the body by the production of diuresis, and that this occurs while the blood is being rapidly resupplied with glucose. In normal animals, too, the extract elevates the blood-sugar values.

The adrenalectomized animal does not lose its water; there does occur an aqueous shift from blood to the liver and muscle, which is apparently of simple, physical proportions. These conditions cannot be considered as presenting an emergency situation. The peripheral cooling and general fall in body temperature which set in following adrenalectomy would necessarily result in circulatory stasis and escape of fluid from the vessels into the surrounding tissues. The carbohydrate deficiencies which occur in the adrenalectomized animal are profound and indeed sufficiently critical in themselves to cause death; they represent a mortal loss of indispensable oxidative materials from the body, and not merely a redistribution of fluids within the tissues. Our data compel adherence to our first-proposed theory of the chief or prepotent function of the adrenal cortex—that of the regulation, in coöperation with other tissues or secretions, of normal carbohydrate metabolism in the organism.

SUMMARY

There is a shift in water balance in the adrenalectomized animal which occurs with the development of symptoms of insufficiency: the liver particularly and also the muscle show increased hydration while the blood becomes dehydrated. The organism as a whole (rat) nevertheless contains more water than normal.

The blood volume commonly (but not in all cases) is reduced after adrenalectomy; in 12 experiments there was an average of 20 per cent below the normal.

Intraperitoneal injections of saline or intracardial injections of saline or gum-saline solution readily restore the circulatory volume, but without affecting the general condition or survival period of the adrenalectomized animal.

The liver glycogen becomes practically depleted, the blood glucose very critically reduced, and the muscle glycogen markedly decreased in adrenalectomized guinea pigs, cats and marmots. In rats there is a marked reduction of liver glycogen.

Emotional excitement and adrenalin injection are practically without effect on the blood glucose of adrenalectomized cats tested at various time intervals after operation while the animals still appear in good health.

Sodium lactate and glucose injections result in no significant glycogen storage in adrenalless animals compared to normal controls. In cases of long-continued, twice-daily injections of glucose during the period following adrenalectomy, there also occurs very little storage of glycogen in the liver.

There is no water lack in adrenalectomized animals with symptoms of insufficiency. The carbohydrate loss alone, however, is sufficiently critical to cause death. Death in convulsions is typical of hypoglycemia, and not of a low blood volume.

The results do not support a proposed circulatory theory of the function of the adrenal cortex. They do offer further strong evidence in favor of the carbohydrate theory of cortico-adrenal function.

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THE OVARIAN CYCLE AND THE ADRENAL GLANDS

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Stilling (1898), Kolmer (1918) and Riddle (1923) have reported that the adrenal glands are hypertrophied at ovulation in the frog, the mole and the pigeon. Anderson and Kennedy (1932) noted that the absolute and relative weights of the adrenal glands of virgin rats are greater at estrus than at diestrus, and advanced cytological evidence to support these findings. Lewis (1923), del Castello (1928) and Schiffer and Nice (1930) reported that the estrous cycle of the rat was not altered by adrenalectomy. On the other hand, Kitagawa (1927), Wyman (1928) and Martin (1932) found that adrenalectomy resulted in the complete or partial suppression of the estrual rhythm.

Our findings, as already briefly reported (1933), corroborate the observations of the last-named observers—the estrous cycle in our adrenalectomized rats was completely inhibited in 19 out of 22 cases.

Connor (1931) and Cleghorn (1932), on injecting cortico-adrenal extracts in rats, observed no alteration of the cycle other than suppression. Cleghorn's extracts, it is to be observed, were admittedly toxic. We were apparently the first to observe that cortico-adrenal extract is able to restore promptly the normal estrous cycle in animals in which complete inhibition had previously been brought about by removal of the adrenal glands. Martin and Kroc (1933) have since reported briefly on experiments which support our results. They noted that cortico-adrenal extract injected into adrenalectomized rats which had shown complete suppression of the estrual rhythm for two complete cycles brought about resumption of ovarian activity after a lag period during which the animal's body weight was restored to normal. They were able to mate some of these animals successfully.

Since our brief report referred to above, we have further investigated this phase of the adrenal problem. We have considered chiefly the effect on the estrous cycle of *a*, adrenalectomy, and of *b*, cortico-adrenal extract; in the latter group we have tested the effect of extract on normal, adrenalectomized and castrated female rats. Over 140 animals were used in this study.

[1] Grateful acknowledgment is made of aid received in this investigation from the Committee for Research in Problems of Sex of the National Research Council.

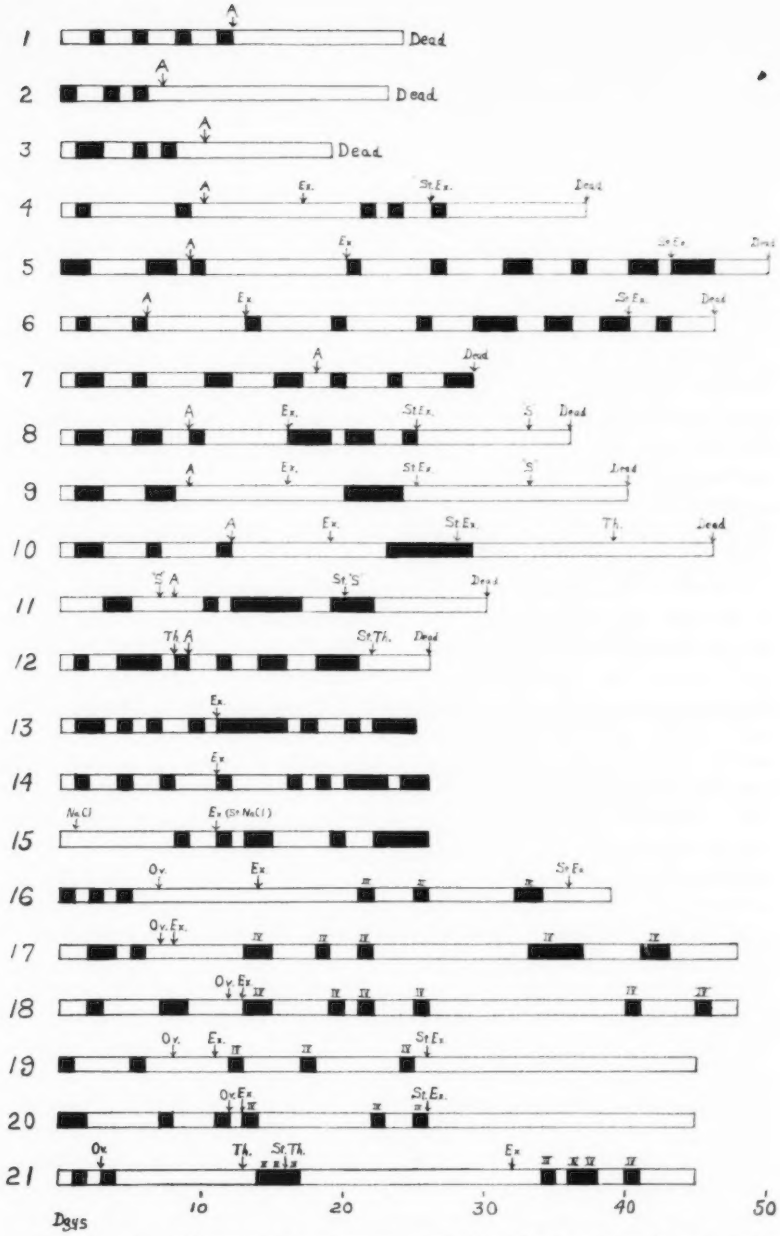
Effect of adrenalectomy on the estrous cycle. Daily vaginal smears were made of all animals used according to the method of Long and Evans (1922). Out of 17 adrenalectomized rats, 12 showed no estrus stages following operation; in three animals the estrous type of smear was present on the day following operation, but did not recur before the death of the animals; one rat showed one ovarian cycle five days after adrenalectomy, with no recurrence; and one animal had an uninterrupted cycle until its death 11 days after operation with a weight loss of 25 grams. All of these animals died showing characteristic weight losses and other symptoms typical of adrenal insufficiency. If the rats showing one estrual stage on the morning following operation are included in the group of "completely inhibited" cases, the results show a suppression of the cycle in 15 out of 17 animals. This series together with our earlier group referred to above thus comprise 39 cases, in 34 of which inhibition of estrus followed adrenal removal. These experiments corroborate those of Martin. Complete adrenalectomy is apparently followed by suppression of the ovarian cycle in the rat.

The fact that in a small percentage of cases the estrous type of smear may be observed after adrenalectomy does not invalidate the above statement. It appears to the authors that the adrenal cortex may furnish a secretion that is essential for the maintenance of normal estrus. It is obviously possible and likely that, in those cases in which some slight ovarian activity persisted, there remained a sufficient amount of accessory cortico-adrenal tissue to maintain ovarian function, and yet an insufficient amount to sustain life itself. It is to be noted that after adrenalectomy the percentage of rats which live indefinitely is, in our experience, larger than the percentage showing the estrous type of vaginal smear. The observation that the ovarian hormone is occasionally able to maintain estrus in adrenalectomized animals is no argument against the possible essential nature of the cortico-adrenal hormone in this respect. Figures 1, 2, 3 and 7 illustrate the effect of adrenalectomy.

Effect of cortico-adrenal extract on the estrous cycle of normal animals. The effect of cortico-adrenal extract[2] on the estrous cycle of normal adult rats was highly variable. In 15 cases, observed for periods of 17 to 44 days, seven animals showed a tendency toward increased ovarian activity; two showed no demonstrable effect, and in six there was evidence of inhibition of the ovarian rhythm. Figures 13 to 15 illustrate the nature of the positive response when it was obtained. In some cases the effect appeared as an initial acceleration followed by inhibition. In other animals in which no estrous stages were observed prior to extract injection, estrus succeeded its administration.

The effect of cortico-adrenal extract, if any, on the estrous cycle of the

[2] Made in this laboratory according to a modified Swingle-Pfiffner method—see Britton and Silvette, 1931.



Figs. 1 to 21. Diagrammatic representation of ovarian activity in rats under various experimental conditions. A few illustrative cases only are given. Black—estrus; white—diestrus.

A—Adrenalectomized. *Ex.*—Extract treatment begun. *St. Ex.*—Extract treatment stopped. *"S"*—Injection of antuitrin "S" begun. *St. "S"*—Injection with antuitrin "S" stopped. *Th.*—Injection of theelin begun. *St. Th.*—Injection with theelin stopped. *NaCl.*—Saline injection begun. *St. NaCl.*—Saline injection stopped. *Ov.*—Ovariectomized. *III*—Stage III of estrus (Long and Evans). *IV*—Stage IV of estrus (Long and Evans).

rat thus remains doubtful. The variable results, if not entirely attributable to chance, may be due to differences in animal response, or to variable potency of the extracts injected. The technique of preparation of the extracts may conceivably allow optimum retention of the life-maintaining hormone, however, and little only of the sex-stimulating factor.

Effect of cortico-adrenal extract on the estrous cycle of adrenalectomized animals. In no case did cortico-adrenal extract fail to restore the estrous cycle in adrenalectomized rats which had been partially or completely inhibited following adrenalectomy. Dosages of from 1 to 1½ cc. per 100 grams of rat per day were used. In three cases (figs. 5, 6 and 8) extract injection was followed by an immediate resumption of estrus—i.e., as observed on the day following extract administration; while in four others estrus was resumed after a lag period of four days' average duration (see figs. 4, 9 and 10). It is noteworthy that subsequent withdrawal of the extract again resulted in cessation of the cycle (figs. 4, 8, 9 and 10).

Antuitrin "S" and theelin were found to be ineffective after inhibition of the cycles had occurred over a period of several days after adrenalectomy (figs. 8, 9 and 10) with a dosage of 10 units per 100 grams of rat per day. The animals were in good condition at the time that injection with these substances was begun, although it should be observed that they had lost some weight. Both substances were found to be effective when administration was begun 24 hours before operation (figs. 11 and 12). Animals so injected died with characteristic weight losses, however, in from 9 to 22 days. Some of these animals died while exhibiting smears of the estrous type (stages 2 and 3, Long and Evans). In an extended series of cases, in which several different batches of material were used, we were never successful in bringing about estrus in adrenalectomized rats with either antuitrin "S" (extract of pregnancy urine)[3] or theelin, when treatment was begun some time after adrenalectomy.

Effect of cortico-adrenal extract on the estrous cycle of castrated animals. Our attention was first called to the possible effect of cortico-adrenal extract on castrated female rats by the case of one animal which showed smears of the estrous type during extract treatment after castration (fig. 16). Smears of this type were present on 4 out of 22 days of observation. Eleven rats were ovariectomized and after a suitable control period were injected with cortico-adrenal extract. Seven of these animals exhibited smears of the estrous type. Two others showed estrous stages in one instance during 25 and 27 days' observation respectively. Two animals showed no estrous stages following operation. The estrous type of smear observed was invariably stage 4 (Long and Evans), round, scaly and neutrophilic cells being present. Adrenal transplants (4 cases) had no effect.

[3] The theelin and antuitrin "S" used in these experiments were generously supplied to us by Parke, Davis & Company through the kindness of Dr. Oliver Kamm.

In three of the cases showing frequent estrous stages an ovarian remnant or cyst was found on autopsy.

Since in none of the cases referred to above (with the single exception of the one cited) were stages 2 or 3 observed, we were inclined to question any specific action of cortico-adrenal extract on the generative tract of castrated female rats, regardless of the dosage employed (1–6 cc. per 100 grams of rat per day) (figs. 17 to 20). Four castrated females injected with theelin (10 rat units per 100 grams of rat per day) exhibited smears of the estrous type for several days consecutively. One animal (fig. 21) which showed a response to theelin injection exhibited no cells of the estrous type following cessation of treatment, but on the injection of cortico-adrenal extract once more showed smears (stage 4) typical of estrus.

In order to test fully the possible action of cortico-adrenal extract on castrated female rats a more lengthy series of animals was studied, and the effect of extract administration evaluated according to the method for the bio-assay of estrogenic preparations originated by Kahnt and Doisy (1928), and used more recently by Curtis and Doisy (1931). According to the latter authors, a response in 75 per cent of the animals tested constitutes a positive reaction. The test animals were selected as follows: Daily vaginal smears were made for a period of two weeks on rats taken from the cages at random. Only rats showing regular estrual rhythms were selected for operation. Following ovariectomy, vaginal smears were continued for a second two-week period. Of this series, only those rats were selected for testing which showed no estrous stages later than two days after operation. All smears were classified according to the method of Kahnt and Doisy, and only those showing cornified cells, or a mixture of cornified and nucleated round cells, were considered as indicative of a positive reaction.

Two complete series of rats were thus selected. All animals were injected subcutaneously (D'Amour and Gustavson, 1930). The "priming" of Kahnt and Doisy was not practised. The rats in group 1 (20) received 4 cc. of extract, and vaginal smears were made after 24, 48, 52, 56 and 72 hours. In this series at least one positive reaction appeared in 8 animals. As noted above, this result constituted a negative test according to the originators of the method employed. The second series (24) yielded 4 cases exhibiting a positive reaction, thus constituting a second negative test.

It is to be observed that positive estrual responses to cortico-adrenal extract injection occurred in approximately half of the normal, unoperated animals. In ovariectomized animals, furthermore, there was evidence of a positive effect in slightly more than 30 per cent of the cases. The results indicate the possibility that estrogenic properties are contained at least in some degree in the extracts which we have employed.

SUMMARY

1. Adrenalectomy resulted in the complete suppression of the estrous cycle in 34 out of 39 operated rats. Residual or accessory cortical tissues are possibly responsible for the persistence of estrus in a small percentage of adrenalectomized animals.

2. The administration of cortico-adrenal extract to normal rats may *a*, influence the ovarian cycle in the direction of increased activity; *b*, produce no effect, or *c*, result in an inhibition of the cycle.

3. Cortico-adrenal extract restored the estrous cycle of adrenalectomized rats in all cases studied. This occurred either at once or after a lag period of a few days.

4. In numerous experiments with theelin and antuitrin "S," it was not found possible to restore estrus when the materials were administered after adrenalectomy. When injections were begun before operation, however, the cycle was thereafter well maintained.

5. No specific effect of cortico-adrenal extract on the ovarian cycle of spayed adult female rats was demonstrated.

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RESPIRATORY FAILURE FOLLOWING DENERVATION OF THE CAROTID SINUS REGIONS[1]

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In the course of experimental denervation of the carotid sinus regions, several dogs unexpectedly died apparently of respiratory failure. Sudden death following section of the sinus and aortic nerves has been observed by Hering (1927), Koch and Mies (1929), Heymans (1929), and Regniers (1930). Hering (1927) ascribed it to the production of ventricular fibrillation, and Regniers (1930) to cardiac inhibition. Respiratory failure in the dog occasionally follows shortly after removal of the stellate ganglia, even without previous carotid sinus denervation (Cromer and Ivy, 1933). In the cat, respiratory failure occurs within a few hours after section of the vagi and the thoracic and cervical dorsal spinal roots; in kittens, section of the vagi alone sometimes has a similar action (Coombs and Pike, 1930). In rats and guinea pigs, following double vagotomy, death occurs in a few hours, preceded by dyspnea (Giusti and Houssay, 1919). A similar type of death, although more rapid, occurs following vagotomy in the horse (Bressou and Bru, 1926). On the other hand, Cromer and Ivy (1933) found that stimulation of the central ends of the cut vagi will lead to apnea and death after double stellatectomy.

It is generally agreed that respiration is affected reflexly by impulses which are transmitted through the vagi. Until recently, it was currently accepted that the end organs responsible for these impulses were located in the lungs. However, the work of Heymans and Heymans (1926) suggested that end organs located in the cardio-aortic region were also involved. Following the discovery by Hering (1927) that the carotid sinus plays an important rôle in reflex regulation of the circulation, considerable evidence has been presented to show that the end organs of this region exert a marked influence in reflexly modifying respiration as well as the circulation (Moissejeff, 1927; Heymans and Bouckaert, 1930; Koch and Mark, 1931, Gollwitzer-Meier and Schulte, 1931; Schmidt, 1932; Winder, Winder and

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Gesell, 1933; Gemmill and Reeves, 1933; Heymans, Bouckaert and Regniers, 1933; Bouckaert and Heymans, 1933). These recent studies have shown that all the end organs in the carotid sinus and cardio-aortic regions are not susceptible to the same type of stimuli and that their effect on respiration is variable. The work of Heymans and his colleagues has shown that the end organs in these regions exert a tonic inhibitory influence, since severing the sinus and aortic nerves is followed by an acceleration and augmentation of breathing. The respiratory failure which we noted following denervation of the carotid sinus regions would seem to indicate the existence of a tonic influence whose action is directly opposed to Heymans' observations. We decided to investigate further in an attempt to determine the mechanism of the respiratory failure following denervation of the carotid sinus regions.

METHOD. In this study, experiments were carried out on 41 dogs, 2 cats and 2 rabbits. In most of these experiments, sodium barbital was used, $1\frac{3}{4}$ cc. of a 15 per cent solution per kilo, injected intravenously, usually with a preliminary subcutaneous injection of morphine sulphate. In 4 experiments, ether was used, and in one, nembutal. Smoked drum records were obtained of the mean blood pressure from the femoral artery, and of intra-pleural pressure.

After a control record was obtained, one carotid sinus region was denervated as completely as possible. The common carotid and the internal and external carotid arteries were stripped for a distance of 1 to $1\frac{1}{2}$ cm. above and below their junction. All the nerve strands in this area were severed, as well as all the tiny blood vessels, the latter between ligatures. Phenol and alcohol were then applied to the region to complete the denervation. The entire denervation required about 5 to 10 minutes. A similar procedure was then carried out on the other side. If the animal survived the bilateral denervation, both vagi were severed high in the neck near the ganglia nodosa. In two animals the vagi were cut before the carotid sinus regions were denervated. The denervation of the carotid sinus regions, as carried out in these experiments, is more complete than that following the more customary section of the two sinus nerves. Cutting the vagi high in the neck is not necessarily equivalent to vagotomy at lower levels, since some of the afferent fibres in the cervical sympathetic nerves join the vagi high in the neck.

RESULTS. The results are summarized in table 1 and typical experiments are shown in figures 1 to 3. A total of 34 out of 45 animals died following the denervation; one after denervating the right carotid sinus region alone, sixteen after denervating both carotid sinus regions, two after subsequent section of the right vagus, and the remaining fifteen after subsequent double vagotomy.

In all of these animals, respiration failed before the heart stopped.

TABLE 1

Summary

"Respiratory" death in 10 seconds or less	15 experiments
"Respiratory" death in 1 to 16 minutes	19 experiments
No death but temporary apnea	10 experiments
No death and no temporary apnea	1* experiment
Mean blood pressure before denervation	90–200 mm. Hg
Mean blood pressure after denervation finished	60–230 mm. Hg
Mean blood pressure at time of last respiration	0–160 mm. Hg
Change in mean blood pressure from control reading to reading after denervation finished	18 experiments, 0 or ± 10 mm. Hg 8 experiments, −20 to −110 mm. Hg 13 experiments, +20 to +70 mm. Hg 6 experiments, no observations
Change in mean blood pressure from reading after denervation finished to reading at time of last respiration	12 experiments, 0 or −10 mm. Hg 16 experiments, −20 to −180 mm. Hg 11 experiments, no observations

* Respiration slowed after preliminary vagotomy.

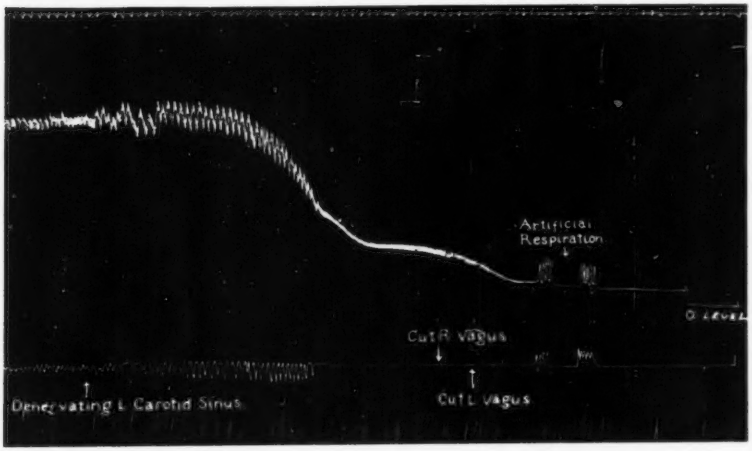

Fig. 1. Record of changes in respiration (lower curve) and mean blood pressure (upper curve) following denervation in experiment 34. Time = 5 seconds.

When breathing ceased, the chest remained in the passive expiratory position, suggesting a failure of the respiratory center to start inspiration (figs. 1 to 3). In 15 of these animals respiration ceased within 10 seconds

or less after denervation, in the remaining 19, respiration ceased from one to sixteen minutes after the denervation. Artificial respiration by manual compression of the chest, with or without 10 per cent CO_2, was tried five times without success (cf. figs. 1 and 2); once, manual compression produced a few gasps; in another experiment, this procedure with 10 per cent CO_2 was followed by restoration of breathing and a rise in blood pressure after a 55 second period of apnea. The resumption of respiration following this procedure may have been a coincidence as, in another experiment, apnea for 65 seconds occurred from which the animal recovered without any intervention.

A temporary apnea, or marked slowing of respiration, following denervation occurred in all but one of the eleven animals that survived. In the exception, the marked slowing occurred following the preliminary vagotomy.

As shown by table 1, the blood pressure level at the time of the last respiration varied considerably in different experiments. In a number of instances, it was depressed only slightly or not at all. An asphyxial rise in blood pressure, usually slight, occurred after respiration stopped (fig. 1).

Discussion. These observations show that denervation of the carotid sinus regions is fraught with danger, at least for anesthetized animals. The risk, in our experience, is "respiratory" death; we have not seen a single instance of "cardiac" death as suggested by Hering (1927) and Regniers (1930).

Respiratory failure is not a constant accompaniment of denervation, and the time of its occurrence varies. Several factors may be responsible for this variability.

The degree of anesthesia and its depressant effect on the respiratory center may be inconstant in the different animals. This fact was demonstrated in the experiments with ether anesthesia. In the three animals in which light ether anesthesia was used, no respiratory failure occurred; while in the one animal in which deeper ether anesthesia was used, a delayed "respiratory" death was produced by the denervation. Differences in the susceptibility of the animals to the action of barbital and morphine may account for some of the variation in the results obtained.

In our experiments, some important reflex pathways to the respiratory center, such as those via the stellate ganglia (Cromer and Ivy, 1933), and the dorsal roots of the cervical and thoracic regions (Coombs and Pike, 1930), were left intact. The reflex effect of various end organ regions is known to vary in different animals of the same species, and the path which the impulses take from them is also inconstant (Katz and Saphir, 1933). In the animals which survived, it is possible that uninterrupted pathways conducted sufficient stimuli to maintain the activity of the respiratory center; whereas, in those that died the stimuli were inadequate.

Denervation of the carotid sinus regions is reported to lead usually to a pressor effect; however, in our experiments, a depressor effect was not uncommon. This latter effect may depend on the use of barbiturates (cf. Lacey, 1932, Vercauteren, 1932). The drop in blood pressure in some experiments appears to depend on the slowed respirations (cf. fig. 2). In

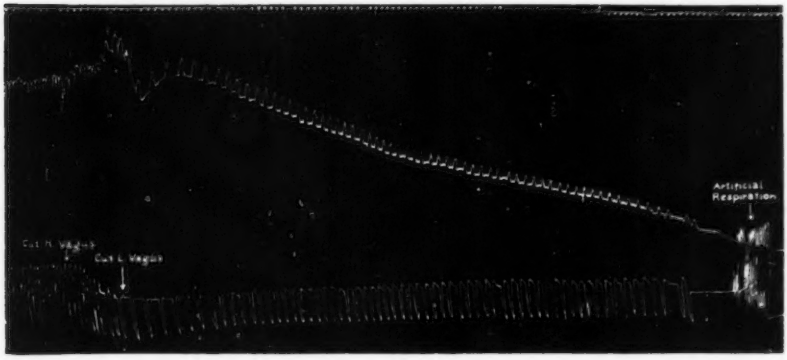

Fig. 2. Record of changes in respiration (lower curve) and mean blood pressure (upper curve) following denervation in experiment 35. Time = 5 seconds.

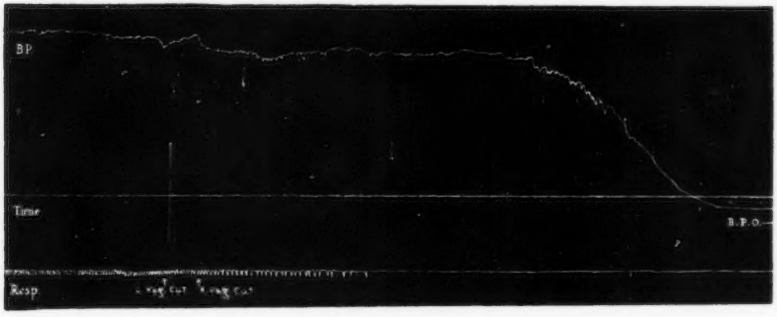

Fig. 3. Record of changes in respiration (lower curve) and mean blood pressure (upper curve) following denervation in experiment 23. Time = 5 seconds.

others the fall in pressure seems unrelated to respiration (figs. 1 and 3). It would seem therefore that the fall in blood pressure is due in part to the mechanical effect of a decreased pumping action of respiration on the venous return, and in part to a depressor effect of the denervation itself on the vasomotor center. Asphyxial changes in the blood subsequent to the slowed respiration may have further depressed the heart and medullary

centers and also contributed to the fall in blood pressure. There can be no doubt that in a number of experiments, the fall in blood pressure to low levels contributed to the depression of the respiratory center, and helped to bring about respiratory failure (cf. fig. 1). This is not the essential factor, however, as respiratory failure often occurred with the blood pressure at or near its normal level (cf. fig. 3). In some cases of delayed respiratory death, a vicious cycle was established, the drop in blood pressure being aided by the slowed respiration, which in turn further depressed the respiratory center until respiratory failure ensued (cf. fig. 2).

While the possibility cannot be excluded that the "respiratory" death immediately following denervation is due to the stimulation produced by cutting the afferent paths, it is our impression that the cessation of respiration is brought about by interruption of pathways normally exerting a tonic stimulating influence on the respiratory center. It is unlikely that delayed deaths could be due to a bombardment of the respiratory center by afferent impulses. Mann (1918) has shown that maximal stimulation of the vagi is followed by death due to respiratory failure only when the animal is very deeply anesthetized. Cromer and Ivy (1933) found that respiratory failure, in stellatectomized dogs, required stimulation of the central end of the cut vagi for an average period of five minutes.

SUMMARY

The results of these experiments indicate that a stream of impulses normally is transmitted from the carotid sinus regions and other end organs, via the vagi, to the respiratory center. Apparently, these impulses help in maintaining the activity of the center. Removal of these impulses by denervation leads to respiratory failure when the respiratory center is depressed. In some instances after denervation of the carotid sinus regions and double high vagotomy, variations in the path taken by impulses from the lungs and cardio-aortic regions may leave enough impulses to maintain the activity of the respiratory center and prevent death. The results suggest that the respiratory automaticity is to a large extent dependent on the functional integrity of the end organs of these regions and the pathways from them to the respiratory center.

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THE PRESSOR AND OXYTOCIC CONTENT OF THE HYPOPHYSIS OF RATS UNDER VARIOUS CONDITIONS

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The purpose of this investigation was twofold: first, to throw more light, if possible, on some of the very obscure functions of the posterior lobe of the hypophysis in the living organism by subjecting rats to various experimental conditions and determining the effect of such treatment on the hormone content of the postpituitary gland; and, secondly, by a biological assay of both the oxytocic and pressor principles of the gland under these conditions, to obtain further data on the unitary or multiple origin of these principles (1).

The only work on the hormone content of the rat's posterior lobe which we have been able to find in the literature is a paper by Pak (2) dealing with changes in the content of oxytocic principle under different conditions such as thyroidectomy, feeding with thyroid, administration of mercury and arsenic, exposure to x-rays, and stimulation of the cervical sympathetics.

In our work we have studied the effect of fasting, adrenalectomy, forced muscular work, fighting for several hours, feeding on a diet poor in water, and intraperitoneal administration of large amounts of physiological sodium chloride solution.

EXPERIMENTAL. Albino rats were used throughout these experiments. For the determinations of the hormone content of the pituitary gland, the animal was killed under ether anesthesia and the whole hypophysis was ground up finely in an agate mortar with M/20 NaH_2PO_4 according to Pak. Instead of transferring the resulting mixture to an ampule, however, we found it more advisable to place it in a calibrated centrifuge tube and dilute it to 4 cc. with M/20 NaH_2PO_4. The tubes were then stoppered loosely with cotton, sterilized in boiling water for 10 minutes and kept in the ice box until used for the assays, which were always carried out within 48 hours after removal of the gland.

For the estimation of the pressor constituents, cats were used either under phanodorn anesthesia, or after decerebration and destruction of the

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upper part of the spinal cord. In the case of administration of small amounts of pressor substance an interval of 30 minutes between injections was found sufficient, which is in agreement with Swanson (3). The oxytocic determinations were carried out on the uteri of virgin guinea pigs in a chamber of 50 cc. capacity. In the assays the extracts of the hypophysis of the experimental animals were compared with extracts made from the pituitary of normal rats and with the standard pituitary powder.

Pak mentions that the gland's content of the oxytocic principle is largely independent of the weight of the animals. The extracts of the hypophysis of rats of different weight caused the same increase in the tonus of the guinea pig uterus. We extended these determinations to the content of pressor component as well. Furthermore, in order to obtain absolute values we compared the hormone content in 20 cases with the standard pituitary powder. From our experiments we can state that in rats weighing from about 100 to 300 grams the content of both the oxytocic and the pressor constituent of the hypophysis is remarkably constant. The amount of each per gland was equivalent to about 0.8 unit of standard pituitary powder. A few determinations were made in pied animals with similar results. We also found that there is no difference in the hormone content between male and female rats. In two young animals weighing 30–35 grams, the content of pressor substance was much below 0.8 unit.

We studied the hormone content of the posterior lobe of the pituitary in rats which had been fasted from 5 to 8 days, in adrenalectomized rats, in fighting rats, and in rats after severe forced muscular exercise. The results in all these cases showed that no change in the hormone content of the rat's pituitary occurred.

Seven experiments were carried out on fasting animals and the data show clearly that fasting which caused great loss of weight and even death in two cases had no effect on the content of pressor and oxytocic constituents in the gland. Only in one case was there observed a decrease in the oxytocic principle, but even in this animal the amount of pressor substance was normal. In six of the seven cases studied the ratio of the pressor and oxytocic constituents was 1. The results of the experiments on the bilateral adrenalectomized rats are given in table 1. Three pairs of full grown male rats were stimulated electrically and made to fight from 4 to 6 hours as in the experiments of Uno[2] (4). After the fighting the animals were killed and the pressor activity of these glands was compared with that of glands of normal animals and with the standard pituitary powder. No difference could be observed. Six

[2] This investigator found that fighting and electric stimulation in male rats caused a change in the effects produced by extracts made from the whole pituitary gland. In attempting to explain these experiments of Uno we noted that the isolated rat intestine responds to the pressor principle in a way opposite to that of the intestine of rabbits and guinea pigs. It reacts with relaxation instead of with increased tonus, and the ileum and large intestine do not show greater sensitivity than the jejunum, as is the case with the intestines of the other animals.

albino rats were forced to run and exercise in a continuously revolving drum for 3 to 5 hours. The animals were exhausted at the end of the experiment. Pressor assays were made and in four cases oxytocic determinations were carried out. Only in one out of the six glands was a difference observed.

TABLE 1

NUMBER	WEIGHT		DAYS AFTER ADRENALECTOMY	HORMONE CONTENT—HYPOPHYSIS		NOTE
	At beginning of experiment	At end of experiment		Pressor	Oxytocin	
	grams	*grams*		*units*	*units*	
15	217	203	1	0.8		Killed, body temp. 96.8°F.
9	138	121	9	Less 0.8	Less 0.8	Killed in extr., body temp. 95.8°F.
18	39		10	Both the same as in the control of aeq. weight		Died, treated with cortical hormone for 3 days
7	240	195	15	0.8	0.8	Killed
13	206	190	9	0.8	About 1.0	Killed, body temp. 95.2°F.
27	118	91	7	About 0.4	About 0.4	Died
33	108	94	12	0.8		Killed at first convulsion, body temp. 94.0°F.
34	122	116	19	0.8	0.8	Died
57	180	172	3	0.8		Died
58	178	146	12	0.8		Died

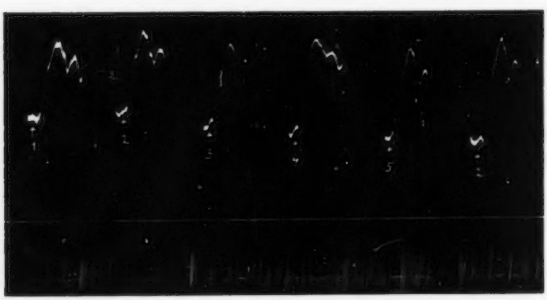

Fig. 1. Blood pressure of a spinal cat. At arrow *1*, 0.5 cc. of the extract of the hypophysis of the fighting male rat 70. At arrow *2*, 0.5 cc. extract of the hypophysis of the control male rat 71. At arrow *3*, 0.5 cc. extract of the hypophysis of the adrenalectomized female rat 58. At arrow *4*, 0.1 unit standard pituitary powder. At arrow *5*, 0.075 unit standard pituitary powder.

We carried out the experiments described below for the purpose of obtaining some data on the well known hypothesis that the pituitary gland plays an important rôle in water metabolism. A recent exposition of this

TABLE 2

NUMBER	WEIGHT		DAYS ON WATERLESS DIET	HORMONE CONTENT—HYPOPHYSIS	
	At beginning of experiment	At end of experiment		Pressor	Oxytocin
	grams	*grams*			
72	190	144	6	Much less than in the control	Much less than in the control
73	168	127	6	Much less than in the control	Much less than in the control
77	177	149	4	A little less than in the control	
78	195	146	6	About 0.16 unit	Much less than in the control
79	252	221	5	Less than 0.36 unit	
80	173	143	4	Little less than in the control	
81	182	133	6	Less than 0.36 unit	
82	198	138	6	About 0.16 unit	Much less than in the control
90	177	168	3	Same as in the control	
91	225	166	7	Much less than in the control	
92	163	125	7	Much less than in the control	

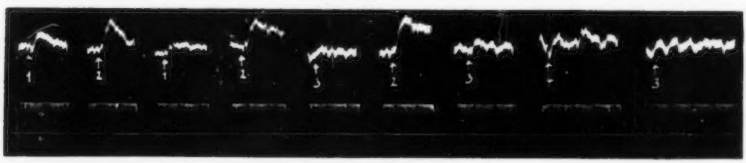

Fig. 2. Blood pressure of a spinal cat. At arrow *1*, 0.5 cc. extract of the hypophysis of the rat 78, held on waterless diet for 6 days. At arrow *2*, 0.5 cc. extract of the hypophysis of the control rat 85. At arrow *3*, 0.5 cc. extract of the hypophysis of the rat 82, held on waterless diet for 6 days. At arrow *4*, 0.02 unit standard pituitary powder.

theory, as also a summary of the earlier work, can be found in a paper by Klisiecky, Pickford, Rotschild and Verney (5).

Our first group of animals, consisting of eleven rats, were fed on the

ordinary mixed diet, but without water and greens, for a period of 3 to 7 days.

The second group of animals (11 rats) received, besides the regular diet, 20 cc. of physiological salt solution injected intraperitoneally three times daily for 3 to 7 days. We found that the pressor activity of the glands of the second group showed no significant change as compared with the glands of the untreated animals, while the amount of this principle was diminished in the dehydrated animals.

It is clear from the table that a slight decrease occurs when the animals are kept for four days on this diet. The decrease, however, becomes very marked after 6 or 7 days. In one case the pressor activity was only about one-fourth that of the normal gland. All the ten animals showed this decrease. In four cases the oxytocic activity was also determined. In every instance it showed a similar large diminution (fig. 3).

Fig. 3. Guinea pig uterus. At arrow *A*, 0.05 cc. extract of the hypophysis of the rat 72, held on waterless diet for 6 days. At arrow *B*, 0.05 cc. extract of the hypophysis of the control rat 74. At arrow *C*, 0.05 cc. extract of the hypophysis of the rat 73, held on waterless diet for 6 days.

Discussion. A significant relationship noted is that in the normal animal the ratio of the pressor and oxytocic constituents is always 1. This means that in the rat's hypophysis these two principles are present in the same proportion as in the International Standard Powder which is prepared from beeves' hypophyses. The absolute amount of both components in the posterior lobe of rats is much higher than in that of cattle. Taking into consideration the measurements of Degener (6) who found that the nervous part of the rat hypophysis weighs 1.00 to 1.24 mgm., and our results, according to which the hormone content of the whole gland is about 0.8 unit, i.e., equivalent to 0.4 mgm. of standard powder, we observe that this unitage corresponds to 2.8 mgm. of fresh cattle posterior lobe. Even if we subtract the amount of posterior lobe hormone which might be found in the anterior part of the hypophysis, the rat's posterior lobe seems to contain at least twice as much hormone per milligram as the posterior lobe of cattle.

Both the absolute and relative amounts of the two components seem to be largely independent of the weight of the normal rat. Moreover, in experiments with the water-deficient diet both hormone fractions were found to be equally diminished. These findings support the unitary origin of these two principles, though naturally more work is necessary before one can draw definite conclusions.

Although the posterior lobe extracts cause very marked changes in metabolism (Geiling and DeLawder, 7) nevertheless significant changes in the metabolism of rats induced by fasting, fighting, forced muscular work, bilateral adrenalectomy and the administration of large amounts of water do not affect the content of pressor and oxytocic constituents in the gland. This may possibly be explained by assuming that under these conditions the amount of hormone produced in the gland exactly equals the amount of hormone secreted plus the amount of hormone destroyed in the gland.

Since the pressor principle usually inhibits the loss of water from the organism, it is probable that during the water-deficient diet, in order to prevent any dangerous decrease of the water content of the blood and tissues, there occurs a large hormone secretion and this results in a reduction of the hormone content of the gland. Naturally, further work must be done to corroborate this possibility.

My sincere thanks are due Prof. E. K. Marshall, Jr., Director of the Department of Pharmacology and Experimental Therapeutics, and also Professors E. M. K. Geiling and A. Grollman for their help and criticism.

SUMMARY

1. The normal rat hypophysis contains about 0.8 unit of the pressor and oxytocic principles. The amount of both principles is largely independent of the weight of the animal. The ratio: $\frac{\text{pressor principle}}{\text{oxytocic principle}}$ was always the same as in the standard powder.

2. In the hypophysis of rats kept on a diet deficient in water for a period of 5 to 7 days, there is a large decrease in the content of both the pressor and oxytocic principle.

3. Fasting for a week, fighting and electric stimulation, forced muscular work, adrenalectomy, the intraperitoneal administration of large amounts of 0.9 per cent NaCl solution for several days, do not influence the pressor and oxytocic activities of the hypophysis.

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THE EFFECT OF ALTERING RENAL BLOOD PRESSURE ON GLOMERULAR FILTRATION

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That glomerular filtration in the normal kidney is a function of arterial blood pressure and the colloid osmotic pressure of the blood is common knowledge. Most experiments on the kidney requiring a record of blood pressure have entailed the automatic recording from some peripheral artery, i.e., the femoral or carotid. Medes and Herrick have shown that the creatinine clearance as measured by Rehberg's method (Rehberg, 1926, 1929) generally parallels the blood flow to the kidney, but no evidence is available to show a relationship between creatinine clearance and renal blood pressure. Blood volume flow may vary independently of blood pressure, and since filtration is primarily a function of pressure rather than volume flow, it becomes of interest to know how altering the renal blood pressure affects glomerular filtration.

METHOD. For this purpose, healthy male dogs were used. Under general anesthesia (Na-ethyl-barbiturate) a solution of creatinine, 0.1 gram per kgm. body weight, was injected into the saphenous vein, the abdomen opened, and after excision of the small intestine, the renal artery of one side cannulated for measurement of renal blood pressure. Cannulation of the ureter of the opposite side permitted continuous collection of urine for determination of creatinine clearance. The common carotid was then cannulated for recording systemic blood pressure and the right femoral vein for introduction of saline solution from time to time during the experiment. A loose ligature placed around the abdominal aorta above the renal arteries permitted constriction to the extent of lowering renal blood pressure.

Creatinine clearance was measured by Rehberg's technique. According to him, the concentration index,

$$C = CrU/CrPl$$

where CrU represents the concentration of creatinine in the urine and CrPl the concentration of creatinine in the plasma, which Rehberg assumes to be equivalent to the concentration in the glomerular ultrafiltrate; then,

$$C \times \frac{\text{vol. of urine}}{\text{time in mins.}} = \text{filtration/minute}$$

We did experiments on only 2 dogs because the results were so similar and definite in each case we thought it was not necessary to do more. Each experiment was divided into eight periods of about 15 minutes each. During the first four periods simultaneous records of blood pressure were made from the carotid and renal arteries without constriction of the latter. At the beginning of the fifth period the ligature was tightened until the flow of urine from the cannulated ureter almost ceased. At the sixth and seventh periods the ligature was loosened, and the pressure completely released during the final period.

Urine collections were closed at the end of each period, the volume measured, and the concentration of creatinine estimated. Blood was

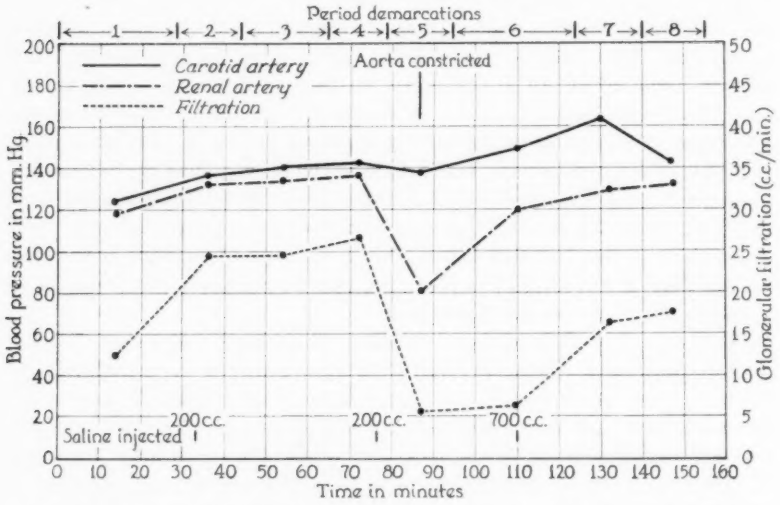

Fig. 1. Variation of glomerular filtration with renal blood pressure. Experiment 2.

collected from the left femoral artery at the middle of each period and the concentration of creatinine in this sample was assumed to represent the average concentration of creatinine in the plasma during the fifteen minute interval. Warm saline solution was introduced from time to time into the right femoral vein.

The relation of renal blood pressure, carotid blood pressure, and glomerular filtration can be seen from the accompanying figure, in which the parallelism between renal blood pressure and glomerular filtration is graphically shown. It may be seen that even before constriction of the abdominal aorta, renal pressure was less than carotid pressure. We found this true in all cases, the difference varying from 6 to 30 mm. Hg in different animals.

Inspection of the curve will show how alteration of renal blood pressure, produced by varying the tension of the aortic ligature, is accompanied by corresponding alterations of glomerular filtration. The difference in pressure, which previous to the constriction had been about 6 mm. Hg at once increased to 58 mm. Hg, aortic pressure remaining nearly constant. Simultaneously, creatinine clearance fell from 27 cc. per minute to 6 cc. per minute. As the ligature was gradually released, renal pressure reverted to its previous level, while creatinine clearance increased to 18 cc. per minute. Response to these changes of pressure was immediate in all cases, thereby supporting Rehberg's theory that blood creatinine clearance is accomplished by filtration rather than by tubular secretion.

SUMMARY AND CONCLUSIONS

Simultaneous records of renal and carotid artery pressure of dogs were obtained under general anesthesia. Urine was collected from the cannulated ureter of the opposite side, and the filtration calculated by Rehberg's method. A ligature around the abdominal aorta above the kidneys facilitated lowering of renal pressure by constriction of the aorta. The glomerular filtration directly parallels the renal blood pressure, while the carotid pressure remains at its higher level.

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ELECTRICAL MEASUREMENTS CONCERNING MUSCULAR CONTRACTION (TONUS) AND THE CULTIVATION OF RELAXATION IN MAN

Studies on Arm Flexors

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That states of nervous irritation and excitement in man are marked by increased contraction in specific muscles has been discussed in previous communications (Jacobson, 1929). This accords with the well-known fact that a considerable proportion of the peripheral nerves of the body (about half, according to Sherrington's estimate) supply the skeletal muscle system. Various evidence has been accumulated, by laboratory as well as by clinical methods, that relaxation can be cultivated in man to counteract nervous excitement. The purpose of the present studies is to investigate this subject with a new, precise method of measurement.

We require a record which indicates tonus or contraction in a muscle or muscle group and the variations from moment to moment during a prolonged period such as half an hour. Whatever apparatus is employed must be interchangeable from person to person, yielding quantitative results of comparable character. Extreme sensitivity is indispensable and there must be a zero line on the record which clearly represents the complete absence of muscular contraction as distinguished from other relatively straight lines due to a slight steady contraction (tonus). Mechanical devices have not yet been developed to effect this nice discrimination; furthermore, they do not seem quite suitable in the intact human subject because they cannot be attached directly to the muscle fibers and because attachments to adjacent or overlying tissues introduce various errors.

These considerations emphasize the need of finer electrical methods for the detection and measurement of muscle contractions, supplemented when useful with mechanical devices. Such procedures have previously been reported (Jacobson, 1930a, 1930b, 1931) and will be further described in the present communications. Six years of experience with the present methods of electrical measurement have convinced me that they are not alone delicate but also sufficiently accurate (see appendix), although certain further improvements are planned.

General methods. The amplifier described in a previous article (1931,

fig. 1) is here used with no filter or condenser on the output terminals, which are connected directly with the input terminals of a string galvanometer (Sanborn). Tension of the string is regulated from time to time during an experimental period so as to keep the excursion of the string shadow approximately 1 cm. per millivolt impressed upon the string terminals when no external string resistance (or external shunt) is present. During measurements of muscular states, a resistance is in shunt across the string terminals, generally of 1000 ohms.

The quest for voltage sensitivity needs to be directed, not so much toward increased amplifying efficiency as toward stability of base line. Therefore, the apparatus was at first removed to a farm, later to a university structure from which the A.C. lighting source could be shut off. Each tube and transformer in the first two stages was encased in permalloy. Finally the apparatus was reinstalled in the physiological laboratory, in a room supplied only with direct current, but adjacent to other rooms supplied with alternating current. Here the amplifier and batteries are contained in a box made of "Armco" iron, 45 inches x 24 inches x 18 inches (height), all 6 sides $\frac{1}{2}$ inch thick, constructed by the Western Electric Company. To increase magnetic permeability, the box was annealed at 1700°F. for four hours and allowed to cool gradually. According to the tables of Scott (1930), with whom I consulted on this point, the shielding properties of "Armco" iron $\frac{1}{2}$ inch thick are equivalent to those of $\frac{1}{8}$ inch permalloy for the frequencies here measured. The selection represents a conjecture rather than an accurate determination as to the thickness required. The cover opens on hinges. Its front end is raised and lowered by means of a chain hoist. To insure uniformly good contact, the cover can be screwed down by means of one or two heavy clamps attached to each side, including the hinged side. All surfaces which meet when the cover is closed have been carefully milled.

A metal box contains the subject and the couch on which he rests. The inside dimensions are approximately 77 x 34 x 40 (height) inches. The sides and bottom are made of soft iron, $\frac{1}{16}$ inch in thickness, but two layers of the metal are used with an intervening air space. Plates of the metal are carefully soldered at all adjoining points to insure electrical conductivity. I have found that under our conditions this box can be used without cover, yielding satisfactory shielding.

A somewhat similar but covered box houses the string galvanometer assembly, including batteries. Tests have not yet disclosed whether this box is indispensable.

For all of the present records the potentiometer of the amplifier has a constant setting. At this setting, the string deflects 3.5 mm. per microvolt impressed upon the electrodes within a certain frequency range (see appendix). Notwithstanding electrical interferences from external sources

and a certain instability internal to the apparatus, the string shadow nevertheless produces an approximately straight line on the moving photographic paper when a short-circuiting wire is across the input of the amplifier. The only irregularities are short vertical lines of high frequency, generally extending not more than 1 mm. above and below the line of zero potential. (Allowance must of course be made for the width of the string.) So far as I know, measurements of such slight transient voltages have not been reported from any physiological laboratory prior to the present apparatus.

During the investigations of the present article, the electrodes are of platinum-iridium wire, gauge 24. The electrode ("positive") connected with the grid of the first tube is inserted about 1 to 2 cm. into the right biceps-brachial muscle at a level about 6 to 8 cm. above the other electrode ("negative"), which is inserted subcutaneously in the elbow pit.

Under these conditions, if the resistance in shunt across the string terminals is set to effect a suitable voltage sensitivity, while the right arm of a trained subject, previously at rest, is flexed and then again relaxed, the string shows corresponding changes from quiet to marked vibrations, which continue so long as flexion is maintained and cease thereafter. If the movement is microscopic in extent, as shown by a lever system suitably attached, the voltage is relatively low (Jacobson, 1930b). Such observations make obvious that there is at least in general a certain correspondence between degrees of muscle contraction and magnitudes of the action-potentials in the wires inserted in the muscle (see Fulton, 1925b).

When the muscles contract to such extent that the potential differences exceed 7.1 microvolts on either side of the zero line, the string shadow shoots off the camera face and escapes photography. Accordingly in most of the tests described in this communication, values above 7.1 microvolts are set down as 7.1+. However, toward the end of this investigation, the shunt resistance box was rendered accessible by placing it next to the galvanometer control box. This makes it possible quickly to increase the shunt resistance, when necessary, so that the string shadow excursions can always be measured, excepting only when the string has been relatively quiet but then shoots off so suddenly that the operator is unprepared to make the change.

In the present investigations, individuals relax under quiet, but not under sound-proof conditions. As a rule the room was fairly free from disturbances due to external sources. When unusual noises occurred, with the subject in the circuit, the string frequently showed increased vibrations, evidently due to subjective reactions. Accordingly tests were generally not conducted when noises were excessive. The experimentor likewise observed precautions to be quiet and to avoid arousing the subject by exciting his attention. Although disturbances affecting the subject or the apparatus directly were not on all occasions completely eliminated, the

errors in the records may be regarded as unusual and as being averaged out in the long run without invalidating the results.

The subject rests upon a canvas cot or a "studio" couch. Since the latter is more comfortable, it would tend to favor relaxation. Therefore with each individual either the cot or the couch was used exclusively (with rare exceptions) throughout the investigations.

After the skin as well as the electrodes have been sterilized with alcohol, they are permitted to dry while exposed at room temperature. The subject then lies down and the electrodes are quickly inserted. Care is taken that he keeps his eye-lids open and converses until the moment arrives for him to begin to relax. This brief interim permits the slight pain due to insertion of the wires to subside. The subjects generally agree that no noteworthy interference with rest results from the continued presence of the wires in the tissues. In my personal experience I have no feeling from them at all during periods when the string shadow indicates complete relaxation. It is only when the muscles are more or less tense that I can at times detect a slight sensation. This agrees with observations previously made that during extreme relaxation, pain is notably diminished (Jacobson, 1925; Miller, 1926; Freeman, 1933).

After the string tension has been set for an excursion of 1 cm. per millivolt, a photographic record is secured while the amplifier input leads are short-circuited by means of a switch. The operator notes the excursions of the string shadow and satisfies himself that they do not exceed 1.0 to 1.5 mm. in either direction from zero. Large excursions such as 1.0 cm., however seldom, indicate that something is wrong. They may occur for a period after the amplifier has just been turned on, after the A-battery has been freshly charged, after heating to solder certain portions of the circuit, after sudden mechanical shocks, or if a tube is defective. Such sources of disturbances have been mentioned previously. With care they can be eliminated.

If we compare the record made upon short-circuit in this manner with a record made under the same conditions excepting that the electrodes are not inserted in the subject, the results are generally the same; but under exceptional conditions the former arrangement shows increased disturbances, evidently due to the subject acting as a sort of antenna. Therefore I have made the short-circuit test after the subject has been connected.

The excursion of the string shadow is standardized by throwing a double-throw switch, thereby impressing an oscillating potential of 1.0 microvolt on the input leads to the amplifier. Upon opening a second switch, the subject is included in the circuit, but the calibrating potential now is connected in series with the "positive" lead from the subject.

In the above steps, then, we secure in about one minute a photographic record of the string excursions during short-circuit of the amplifier input, as

well as a record of the excursion due to one microvolt impressed on the amplifier directly or through the subject. The magnitudes of the excursions during short-circuit, if expressed in microvolts, are one measure of the error of the instrument and must be subtracted from the magnitudes recorded during measurements of potential differences in the electrodes.

Most frequently the record is taken for 30 minutes while the subject reclines. A continuous photograph covering this period would be feasible if the speed of exposure could be limited to slow rates such as three to nine feet per hour. To effect this purpose, reducing gears were constructed and the intensity of the lamp-light was variously adjusted with a rheostat, while the slit through which light enters the camera-box was suitably diminished. Nevertheless I failed to get satisfactory photographs. Accordingly it was decided to run the paper at about one inch per second. For economy, the motor which turns the roll of paper is started and stopped at regular intervals. In the earliest records, the paper is exposed for five or ten seconds or more every minute or so. This was effected through switching the motor on and off by hand. But in almost all records of the present series, a device is used which automatically turns the motor on and off regularly three times per minute. Each exposure lasts about two seconds, so that the interval between exposures is about eighteen seconds. The device consists of a clock which conveniently has a stop-switch somewhat like that of a stop-watch. In place of hands is a vertically revolving disc about 8 cm. in diameter, with three short platinum wires set radially at equal distances apart. A cup containing mercury is arranged below the disc so that each wire makes contact as it passes through the liquid metal. This closes a relay circuit so that the motor starts and stops at intervals as desired. In this way a photograph is secured, about 2.4 inches wide and perhaps about 20 feet long. It contains 90 sections per half-hour.

The records contain so may lines that it seems impractical to measure them all. Accordingly for graphing, we select the maximum vertical line in each centimeter of record, exclusive of the lines due to the pulse. To save time in making the readings, we measure (mm.) from the point of zero potential to the end of each line. That is, throughout each record, we read only on one side of the zero line. (A slight inaccuracy is introduced by failing to measure the length of the entire line, because the string shadow does not always vibrate symmetrically with reference to the zero axis. But since the deviations from symmetry are not great as a rule and since we follow the same procedure in dealing with all the records of the present investigation, I believe that the inaccuracy mentioned may be neglected.) The linear lengths, so measured, minus one half the short-values, indicate the maximum microvoltages per centimeter impressed during muscular contraction upon the electrodes. Values of action potentials so measured will be called V_m per cm. Where such values exceed 7.1 microvolts, if the extent

of the line shows that the string-shadow shot beyond the camera face, we shall for the present set them down as 7.1.

Students not trained to relax. Relaxation in college students was tested with two groups of both sexes, mostly males. Group 1, eight males and two females, were undergraduates ranging in age from 18 to 25. They were members of a course in elementary physiology and volunteered their

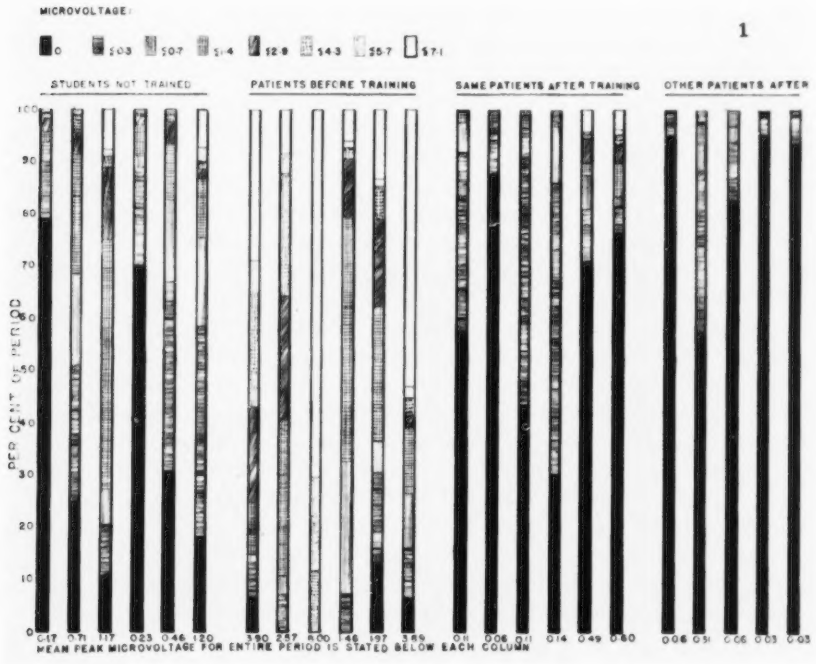


Fig. 1. In the column for each subject, the various markings indicate during what percentages of the half-hour period the microvoltage (V_m) is less than or equal to certain values (see key). For example, the first column shows that this subject was relaxed 79 per cent of the half-hour and at least approximately relaxed 93 per cent of the half-hour. Below each column is stated the mean peak microvoltage (mean V_m) for the same subject and period.

services. Group 2, six males, included three undergraduates within the above-mentioned age limits and three graduates who were 28 to 30 years old. Members of group 2 were paid by the hour for their attendance.

The students were not informed as to the purposes of the investigation. In all instances they clearly understood in advance that they were to keep their eyes closed during the entire period of test and were not to speak or

be spoken to. Otherwise the only instruction given to members of the second group was to relax all muscles as completely as possible. In some of the cases of the first group, a slightly different wording was used in the instruction. This will be considered later.

Each student was tested as described on two days about a week apart. The records of the second test of the second group are represented in figure 2, and also in figure 1 under the heading, "Students not Trained." No student achieves and maintains complete relaxation of the arm muscles during the entire thirty minute period. The closest to this is achieved by one student who shows approximately zero action-potentials for 79 per cent of the time. For an additional 14 per cent of the time, the action-potentials are very slight (not in excess of 0.3 microvolt); his relaxation is practically complete for a total of 93 per cent of the time. This subject is a graduate student of sociology, aged 29, who states that he is fairly well except that he has had chronic constipation and occasional mild backache for about two years. He adds that he has tried to train himself to relax. "I make up my mind, 'Now I'm going to relax!' Of course it does not always work."

For the five other subjects relaxation is approximately complete for smaller percentages of the time, ranging down to 20.2 per cent. It is striking that a presumably "normal" subject, requested to relax completely, fails to do so during 24 out of 30 minutes, notwithstanding that conditions are quiet and the couch very comfortable. The one with this lowest record is a graudate student of English who states that at times he is "high strung" and excited. Occasionally he manifests slight diarrhea. These findings suggest that we must use caution in classifying subjects as "normal" or "not nervous" merely because they have not consulted a doctor.

The next to the lowest record is made by a college Junior of 23 years, who relaxes approximately completely for only 51 per cent of the period. He states that he is well and is not "nervous" or "excitable." Another student (Law, aged 30) whose record for approximately complete relaxation is relatively low, remarked after the period, "I tried hard. Sometimes relaxing becomes hard work." According to experience in teaching relaxation (Jacobson, 1929), "trying hard" to relax defeats its own end, resulting in partial failure.

Every record from these untrained students shows one or more moments of tension exceeding 5.7 in microvoltage (see fig. 1). Such irregularities are unavoidably obscured in figure 2 owing to the method of averaging. As will later be seen, they do not commonly occur in the records of certain highly trained subjects.

Under each column in figure 1 is stated the mean peak microvoltage (mean V_m per cm.) for the same subject during the half-hour period. Roughly speaking, and with exceptions, the mean V_m varies inversely with

the percentage of the period characterized by complete or almost complete relaxation (microvoltage $\leqq 0.3$).

A brief way to sum up the results for an entire record, then, is to state the mean peak microvoltage. This is shown in table 1 for six students, in order to determine whether the first and second tests differ greatly in results. As will be seen, the mean V_m for two subjects is the same in both tests, for two increases and for two decreases. The average for all subjects together, except J.B., increases. Furthermore, at one or more points on the records of each of the six subjects during the first test, as well as the second, the string-shadow passes off or nearly off the camera face. Accordingly, it is evident that although some adaptation doubtless tends to

TABLE 1

Mean peak microvoltages for group 2 (6 students not trained)

	SUBJECTS						
	T.N.	S.D.	J.B.	C.D.	A.L.	C.R.	All except J.B.
1st test	0.26	0.40	6.29	0.23	0.46	0.69	0.40
2nd test	0.17	0.71	1.17	0.23	0.46	1.20	0.56

TABLE 2

Mean peak microvoltages for group 1 (9 students not trained)

	SUBJECTS									
	H.M.	N.L.	J.P.	P.F.	E.B.	F.N.	S.T.	J.I.	M.C.	All, except J.I. and M.C.
1st test	0.16	0.16	0.44	0.46	1.06	1.07	1.07	2.03	3.34	0.63
2nd test	0.64	0.43	0.11	0.43	0.21	1.26	1.24	0.49	0.29	0.61

occur, resulting in increased relaxation, yet as a group the records give a fairly similar showing for the first and second tests.

Tests on group 1 differ from those on group 2 in that two (in place of one) half-hour tests were made on each of two days about a week apart. For the second half-hour period on each of these two days, the request was to relax completely, as above-mentioned. For the first half-hour period the word "relax" was omitted from the request, which was in some cases to avoid marked movement or to avoid violent movement and in other cases to lie as quietly as possible. As a rule these untrained subjects reported afterwards that this wording meant to them the same as to relax, although one or two subjects said that a more conscious attempt to relax followed the request which specifically included the term "relax." In the present paper, we consider the records only of the second half-hour periods. From

these records are computed the mean microvoltages as shown in table 2. Comparing the results for the first and second tests of these nine subjects, the mean V_m for three subjects remains practically equal, for two increases and for four decreases. (Both of the subjects whose mean V_m for the first test exceeds 1.4 believe that they are not "nervous"; but J.I. stated that on this particular occasion "she might be nervous, since she just came from a final examination.") The average of means for all subjects, excluding two, is approximately equal for the first and second tests.

In the first tests, the string shadow passes off the film ($V_m > 7.1$) at one or more points in all records but 2. (The exceptions are H.M., with maximum $V_m = 1.1$ and N.L., with maximum $V_m = 5.7$.) In the second tests there are twice as many of such exceptions (E.B., with maximum $V_m = 0.9$; M.C., with maximum $V_m = 1.4$; N.L., with maximum $V_m = 3.7$, and P.F., with maximum $V_m = 4.3$.) According to the characteristic thus illustrated, the second tests show somewhat greater relaxation of the subjects as a group than the first tests. For these nine subjects, as a group, adaptation apparently results in a somewhat greater lowering of microvoltages than for the group previously considered. This difference in the two groups may be ascribed to a somewhat greater relaxation in some subjects of the second group following a preliminary half-hour rest. Nevertheless also for the second group the showing is fairly similar for the first and second test-periods. For all subjects in each set of nine tests, excepting only two tests, the average value falls below 1.4 microvolts.

In summary, the foregoing tests on two groups of unselected students, aged 19 to 30, generally show mean $V_m < 1.4$. For all the students in both groups, the average of mean V_m is 0.90. Considering only those students (12) for whom mean $V_m < 1.4$ in both tests, the average of means is found to be 0.57 microvolt. It is important to note that for one student out of each five tested, mean $V_m > 1.4$ in one of the two tests.

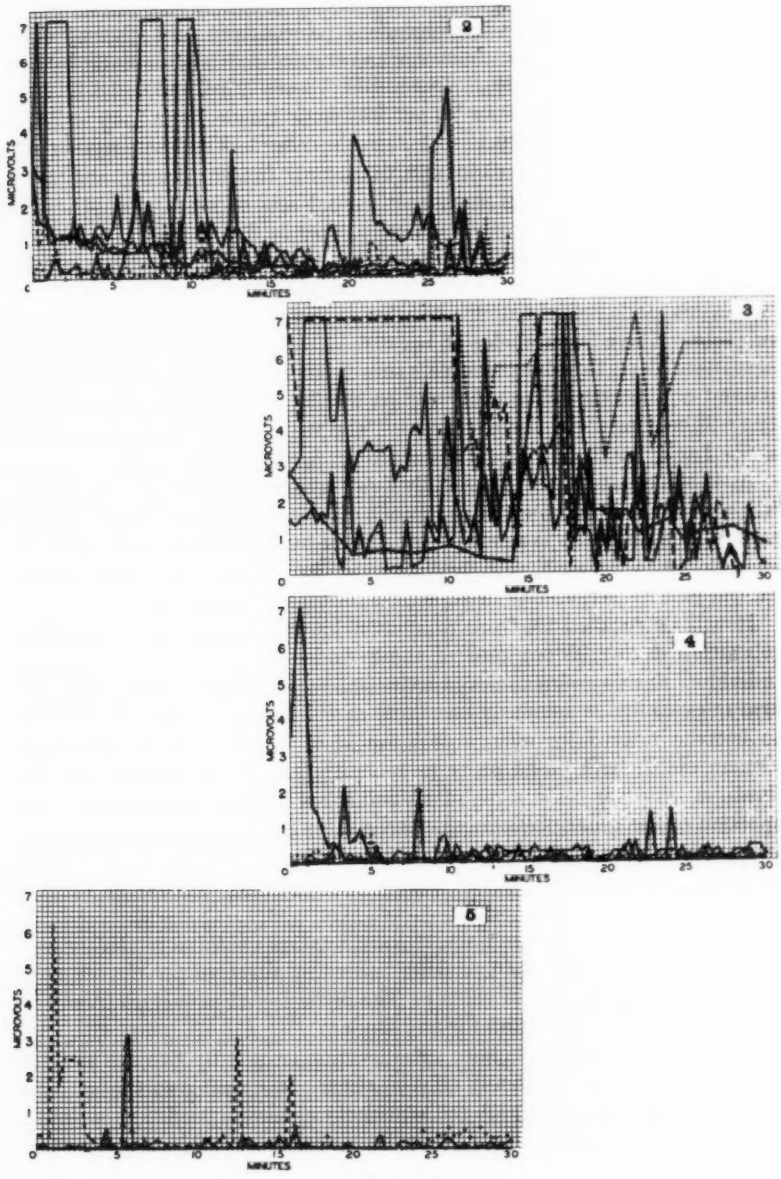
Patients before training to relax. In the present investigation one or more

Fig. 2. Curves for six "normal" students, not trained to relax. Microvoltage (V_m per cm.) is plotted against time. The instruction given is to relax as fully as possible. (In this and the following figures, values ≥ 7.1 microvolts are plotted as 7.1.)

Fig. 3. Curves for five patients with certain neuromuscular disorders before training to relax. As will be seen from comparison with figure 2, the microvoltages for this group are considerably higher than those for the "normal" students.

Fig. 4. Curves for the same subjects as shown in figure 3, but after training to relax. The microvoltages are considerably lower than before training. They are lower also than those for the students shown in figure 2.

Fig. 5. Curves for five other patients after training to relax. No electrical records had been made with these subjects previously. Since the curves on the whole are as low as those in figure 4, it is evident that repetition of tests is not the cause of the progressive decrease in microvoltage generally observed during training to relax.



Figs. 2–5

records were made of each of 50 individuals with disorders that are classified as neuromuscular hypertension, chronic or intermittent, and other nervous or fatigue states, with or without marked chronic colitis or vascular hypertension (cf. Jacobson, 1929). In records of twenty patients who had received no training, the mean V_m per half-hour ranges from 1.46 to 7.1+ in half the cases and from 0.14 to 1.29 in the other half. These results will be discussed later. Complete sets of records before, during and after training were taken in 12 instances; partial sets of records in other instances, single records at one stage or another in the remaining instances. Concerning the central problem of this article, whether relaxation can be cultivated in man, the results in general are uniform.

Records made before training to relax are represented in figure 3 for five subjects. These five are selected because V_m averages higher than for other nervous subjects for whom complete sets of records had been made with the present equipment. One other record should be included in this group, but will be considered alone later on. The values set forth in figure 3 are arrived at by methods already described for figure 2 (see legend).

As is strikingly evident, the individuals with neuromuscular symptoms whose records are represented in figure 3 exhibit an almost complete failure to relax. This point is brought out clearly by comparison with figure 2. In addition to higher V_m for the "nervous" individuals, their photographs exhibit greater irregularity in V_m during the half-hour periods.

It will be recalled that one student achieved approximately complete relaxation during 93 per cent of the period. In contrast therewith, no individual in this group achieves approximately complete relaxation in the arm muscles for as much as one-third of the period. One subject fails to become relaxed for even a fraction of a second. She is a married woman of 37, with blood pressure generally above 220 systolic and 140 diastolic, showing some signs of vascular and kidney degeneration. Inspection of the other photographic records for these patients discloses that they seldom relax during the test for even a minute; the longest period that any one of these six subjects remains continuously relaxed is about 4 minutes.

Records of the six subjects under consideration exhibit a relatively high mean V_m, ranging from 1.46 to 8.00. (Similar averages for the students generally fall below 1.30 in value.) The highest of these values is over 72 times the lowest value found for any student.

Five of the six subjects under consideration frequently become so tense that the string-shadow shoots off the camera face owing to the voltages being too high for record with the sensitivity generally used. It is certain in these instances that the maxima set down are considerably too low. Accordingly the mean V_m above stated is unduly low in value.

After training in relaxation. Treatment of the groups of patients herein

described was in practically all cases limited strictly to methods of relaxation alone. These methods have been sufficiently described (Jacobson, 1929). Instruction in the recognition of tension by muscle sensation and in relaxation was given generally twice a week for periods of an hour. In addition, the subject was requested to practise during each day for one or two hours.

Records made after training to relax (generally 4 to 9 months) are represented in figure 4 for the same five subjects as shown in figure 3. It is obvious that the microvoltages (action-potentials) in these subjects after training are much smaller than before training.

The same diminution is seen in figure 1, comparing the columns for the same patients before and after training. The subject who failed to relax for even one moment before training, relaxes approximately completely for 98.8 per cent of the period after training. Whereas before training, the range for approximately complete arm-muscle relaxation in these six subjects is from 0.0 to 30.3 per cent of the 30 minute period, after training the range for the same subjects is 81.0 to 98.8 per cent. Before training, the average of mean V_m for the six subjects is 3.63, while after training it is but 0.26. For the fifteen students in both sets of tests the average of mean V_m is 0.90,—a figure about three and one-half times as great as that for these trained subjects. Furthermore, before training, all subjects but one have moments when $V_m > 7.1$, while after training, four of the six subjects show no excursions beyond 2.0 in microvoltage and seldom any as great as 1.4 microvolt. After training, the other two subjects show some excursions exceeding 7.1 in microvoltage, but they are now relatively infrequent.

As just mentioned, the record of subject A.F. after training shows some relatively large excursions. These and somewhat lesser ones occur irregularly from time to time during the period, between intervals of microvoltage not far from zero. Results for this subject, when included in figure 4 yield a curve more irregular and somewhat higher in microvoltage than the five curves in that graph. This curve belongs in figure 4 but has been omitted because it was found to obscure the curves for the other subjects. However, if the values for this subject are plotted, apart from other subjects, the curve after training is distinctly lower as a whole than before training. This diminution may be clearly seen from columns 12 and 18 in figure 1. Before training, her arm muscles show relaxation approximately complete for only 16.0 per cent of the period, but after training for 81.6 per cent of the period. Before training, the mean V_m per cm. per half-hour is 3.89, while after training, it is but 0.60.

In determination of basal metabolic rates, as is well known, repetition of tests tends in the direction of lower values, at least with some subjects, and is generally attributed to increase in muscular relaxation. Accordingly it seems desirable to set up conditions such as omit the possibility that the record of very low microvoltage is secured because of repetition of tests.

Five subjects were available who had clear histories of severe nervous symptoms but who had been trained to relax. Before treatment one had exhibited chronic spastic esophagus with a background of moderate cyclothymic depression; the second had feared jumping out of the window and had other nervous difficulties in practising his profession; the third stammered severely, especially when under stress; the fourth had frequent loose bowel movements with mucus and passing of flatus, interfering with normal social activities, and the fifth gave a general history of nervous maladjustments and deported himself rigidly. All originally showed outward evidence of restlessness and inability to sit still with limbs persistently relaxed. The above-mentioned symptoms or manifestations, according to the reports of these subjects and so far as could be observed by clinical measures, had in great part disappeared during the course of training to relax; but no electrical records had previously been taken. Here, therefore, the factor of becoming accustomed to the tests does not enter.

Figure 5 represents the first records for these subjects, with one exception where it seemed proper to retake the record, owing to noises in the laboratory occurring during the last twelve minutes of the period. Evidently V_m for these five subjects, for whom no question of habituation enters, are even somewhat lower than for those represented in figure 4. In four of these subjects, the mean $V_m \leqq 0.06$,—whereas in the trained subjects who had undergone previous tests only one is as low as 0.06. The average of mean V_m for these five subjects as a group is 0.10. This figure is but one-ninth of the similar value for the fifteen students. As previously stated, at one or more points in the first record of thirteen out of the fifteen students tested, the microvoltage is 7.1 or more. This is never true of the four trained subjects who were tested only once. The maximal V_m at any moment is respectively 1.1, 2.9, 3.1 and 4.0.

The five subjects whose records before training are represented in figure 3 and after training in figure 4 were tested also from time to time during training. As a rule records during training are intermediate in average microvoltage and in the number of moments of high microvoltage. The curves on the whole become progressively lower with training until a relatively low level is attained. Thereafter records may continue to show slight improvement or may continue on practically the same level; needless to say there are instances when they show the reverse of progress. (In my opinion and according to reports of the subjects, various factors influence the results, as indeed they do at all stages of training: most commonly intercurrent illness, anxiety or grief.)

Discussion. The graphs and tables herein presented concern the question whether persons who are commonly hypertense or nervously excitable —including sufferers from various forms of neurosis, chronic colitis, vascular hypertension and other pathological physiological disorders—can be

trained to relax. As this training proceeds, according to clinical observations, there occurs a certain abatement of symptoms, at least in so far as no unalterable organic pathology exists. The effects of daily rests alone, without training to relax, remain to be determined. Aside from this, the results furnish further evidence that this type of person can be trained to relax.

It was at first surprising to find that of twenty patients with disorders as above stated, ten yielded records in which mean $V_m < 1.4$. Of these ten, at least five had been characteristically restless and nervously irritable. (These five included three who were hypochondriacal to the point of disability, respectively: cardiac neurosis, fatigue neurosis, atypical facial neuralgia, one showed vascular hypertension and one had vague anxieties which incapacitated him. In the remaining five, restlessness and nervous irritability had been less marked. This sub-group included one sufferer from recurrent headache, one from chronic colitis and arthritis, one from insomnia, fatigue and mild colitis, and one from restlessness and hyperemotionality.)

The following considerations suggest why persons even of pronounced neurotic type may present records similar in microvoltage to those from unselected university students: 1. It is common experience that many "nervous" persons are highly variable in their manifestations;—at one time they are relatively calm, at another, excited. We should therefore expect such persons at times to give a record fairly "normal." An example in point is the patient in this group who has a cardiac neurosis and whose mean $V_m = 0.9$. Ten days later a second record shows mean $V_m > 7.1$. 2. According to my clinical experience, some individuals relax well lying down, but become hypertense during activities that involve stress and strain. That is, they fail to relax differentially. While perhaps the majority of "nervous" patients report more or less insomnia, there is a considerable minority who state that they sleep very well. (Training to relax, therefore, has two chief stages: first, general; second, differential.) We should accordingly expect some patients of this type to give fairly relaxed records lying down. 3. Three of those who show records in which mean $V_m < 1.4$ report that during past years they have as a rule rested one or more hours daily. It is conceivable that in these instances the effect of such habits has been to reduce the tension when lying down. But in any event, according to their reports and our clinical observations, they had been restless and over-excitable when sitting and conversing. 4. A record from a particular group such as the right flexor muscles does not necessarily represent the state of contraction in most or all other muscles. When other muscles contract there is likely to occur an increase in the group being tested, as has often been verified. But occasionally in "nervous" patients (and characteristically in subjects who have been trained to relax) such

increase is absent. Although W, suffering from atypical facial neuralgia, habitually shifted all his limbs every few seconds and continued to move his left hand to and from his face during the record, his right arm relaxed so that mean $V_m < 0.7$. Such differential quiet of the right arm muscles is exceptional in nervous persons; but this instance is recounted because it illustrates that the state of the right arm muscles is not necessarily an index of the state of other musculature. Clinical observation suggests that "nervous" patients may relax the arm to a certain extent, while nevertheless showing marked evidences of restlessness and over-activity of the eyes and speech apparatus.

The present investigations have been greatly furthered by the support of the Bell Telephone Laboratories. I am grateful to Messrs. G. D. Arnold, H. A. Frederick and D. G. Blattner for interest as well as for aid on various important technical points.

CONCLUSIONS

1. Apparatus and procedure are described which make it possible to measure practically all degrees of muscular contraction in electrical terms. To effect this, transient potential differences (action-potentials) are recorded to fractions of a microvolt. Degrees and variations of muscular contraction over a prolonged period can be represented in graphic form while the mean value during the period can be expressed as a certain number of microvolts.

2. Apparently healthy persons (college students) attempting to relax completely, commonly fail to do so to some extent. From time to time their arm muscles contract, at least slightly.

3. Certain patients suffering from "nervous" disorder, vascular hypertension or chronic colitis, characteristically yield records showing marked inability or failure to relax.

4. Measurements made on these individuals before and after training show that relaxation can be cultivated. After training, they relax more fully, as a group, than do the untrained college students. The results of daily rests alone, without training, remain to be determined.

5. Low microvoltage is found after training to relax, even if no test has been made previously. Therefore repetition of tests evidently is not the cause of the progressive decrease in microvoltage generally observed during training to relax.

6. Some of the untrained subjects with disorders mentioned show hypertension in some records but fair relaxation in others. They vary considerably until they are trained to relax more nearly habitually.

APPENDIX

CALIBRATION. *A. Method.* Throughout most of the present records the potentiometer of the amplifier, which has 22 steps, is set at step 16, and a 1000 ohm shunt is

across the galvanometer input. With this setting, in order to evaluate string excursions per centimeter in terms of voltages impressed upon the input of the amplifier, standardizing procedures are carried out as follows:

1. A potentiometer is arranged as in figure 6 as a source for voltages ranging from 10^{-6} to approximately 9×10^{-4}. The circuit includes a dry cell and a resistance A so varied that 1 milliampere flows through it, making the drop across points B and C equal to one millivolt. A somewhat similar arrangement divides this millivolt further. The accuracy of the millivolt above-mentioned was checked with a Leeds and Northrup portable potentiometer. The accuracy of the apparatus shown in figure 6 when set to maintain a constant output of one microvolt was further tested by means of a White Potentiometer, with the kindly assistance of Dr. T. F. Young and the error found to be less than 1 per cent. In order to apply this and other voltages to the amplifier, the connections to a commutator on a synchronous motor are so arranged that the direct current can be interrupted sixty times per second, or by turning the switch D, an alternating current at 60 cycles is produced. Interrupting or alternating the current in this way provides a frequency that can be conveniently amplified.

Upon setting the potentiometer to an indicated 10 microvolts D. C. the string deflection is 3.55 mm. per microvolt (fig. 7 A). This value is obtained after subtracting the spontaneous fluctuations in the base-line (fig. 7 B). (To calculate this, the vertical fluctuations over five inches of base-line—evidently a fair sample of the entire photograph—were read with a magnifying glass and the average value determined. A similar method was used to determine the average string deflection in millimeters per microvolt.)

2. Upon setting the potentiometer to five indicated microvolts A. C., the total string deflection per microvolt A.C. is 7.0 (fig. 8). The discrepancy between the above-mentioned values is less than 1 per cent.

3. An oscillator calibrating circuit (frequency approximately 57 cycles; loc. cit., fig. 3) kindly provided by the Bell Telephone Laboratories is first applied as follows: Setting its dial so as to impress one millivolt A.C. directly on the string galvanometer yields a string excursion 1.13 cm. per indicated millivolt (after subtracting vertical values of the base-line; see fig. 9). (The millivolt source used for standardizing the string was found accurate upon comparison with another millivolt source which had been carefully checked with the Leeds and Northrup potentiometer.) Setting it to impress respectively 1000, 100, 10 and 1 microvolt on the amplifier galvanometer circuit with shunts across the string terminals respectively 9, 90, 1800 gave step-down ratios respectively 1:10 (figs. 10 and 11), 1:10 (figs. 12 and 13), 1:12 (figs. 14 and 15). Allowing for these ratios and for the peak string excursion 1.13 cm. per indicated millivolt as above-mentioned, the string excursion for one indicated microvolt yields a value 7 per cent too small. Applying this correction to the excursion observed when the 1000 ohm shunt is used (fig. 16) gives 3.69, which differs from the value stated under caption 1 and 2 above by less than 5.5 per cent.

B. Response to various frequencies. 1. The present amplifier has a fairly flat characteristic for frequencies ranging approximately from 30 to 4000. This has been illustrated previously (loc. cit., fig. 2). The question might arise for some readers how this is possible when the coupling is inductive? —in other words, why is it not necessary to calibrate in terms of voltage per unit of time? The following concise statement on this point is quoted from a letter written by Mr. H. A. Frederick of the Bell Telephone Laboratories:

"The wave form resulting from opening and closing a steady current at equal intervals of time can be represented as a series of separate and distinct components,

one of which is a steady flow (D.C.) and the others are sinusoidal waves of the frequency of interruption or odd harmonics thereof. This can be expressed:

$$\frac{e}{2}(1 + \sin \omega t + 1/3 \sin 3\omega t + 1/5 \sin 5\omega t + \ldots\ldots\ldots\ldots 1/n \sin n\omega t)$$

If by some means the steady component $\frac{e}{2}$ is suppressed while the sinusoidal components are accurately maintained relative to each other both as to magnitude and as to phase, the resultant will be identical with the original except that it will be shifted so as to become symmetrical about the time axis. This is not a matter of amplifier design but is a mathematical truism. Obviously then, the problem as applied to inductive amplifier design is one of maintaining uniformity of response from the frequency of interruption to the maximum frequency component of interest and of maintaining the proper phase relationship over this frequency range. This has been effectively accomplished in the design and hence no time rate enters into its calibration."

2. Having in mind that strings would probably break more often if used at higher tensions, the tension was kept to yield a shadow excursion of 1 cm. per millivolt as previously stated. Unfortunately this leads to a noticeable drop in response to frequencies above 60 (see fig. 17). I have found that the response characteristic can be made fairly flat from zero to about 300 with suitable strings tightened to effect a shadow excursion of about 1–2 mm. per millivolt; but accidents have increased in frequency. 3. As is well known, whatever frequency is impressed, a certain amount

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Fig. 6. Diagram of calibrating apparatus. Provides one microvolt or more D.C., constantly or interrupted 60 times per second; or A.C. at 60 cycles.

Fig. 7. A. Excursion (vertical lines) in millimeters (horizontal lines) when 10 microvolts D.C. is impressed with apparatus shown in figure 6. B. Upon short circuiting the leads to the amplifier, inductive effects due to the motor are evidenced by fluctuations in the base-line. These are subtracted from the excursions in A to determine the excursion per microvolt.

Fig. 8. Five indicated microvolts A.C. Otherwise conditions as in figure 7.

Fig. 9. One indicated millivolt from oscillator calibrating circuit impressed directly on string terminals.

Fig. 10. One thousand microvolts from oscillator circuit calibrating amplifier-galvanometer system with 9ω shunt. In figures 10 to 15 inclusive, the string tension is constantly such that one millivolt indicated on the galvanometer voltmeter yields an excursion approximately 5 mm., as shown in figure 12 C.

Fig. 11. One hundred microvolts. Otherwise same as in figure 10.

Fig. 12. Shunt 90ω; 100 microvolts.

Fig. 13. Ten microvolts. Otherwise same as in figure 12.

Fig. 14. Shunt 1800ω; 10 microvolts.

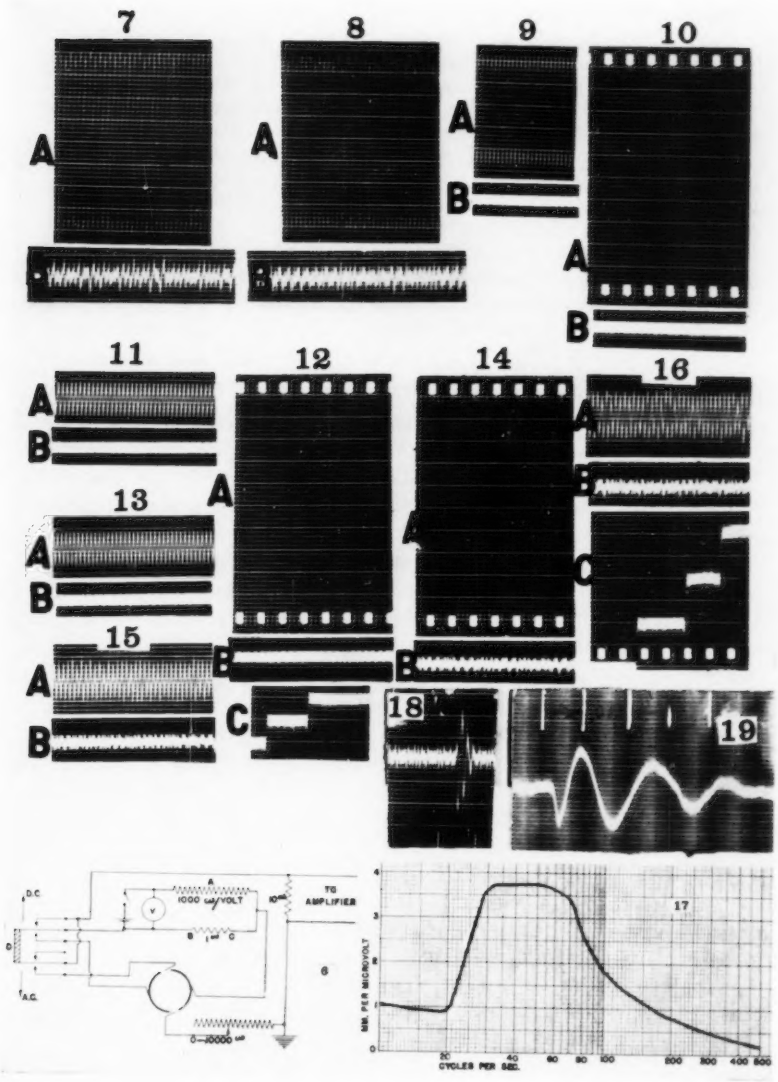
Fig. 15. One microvolt. Otherwise same as in figure 14.

Fig. 16. Shunt 1000ω. A. One microvolt. B. Short circuit across input leads. C. String tension set to yield excursion approximately 1 cm. per indicated millivolt.

Fig. 17. Response of string galvanometer-amplifier system to various frequencies impressed with oscillator calibrating circuit, including also a low frequency oscillator outfit.

Fig. 18. Response of galvanometer amplifier system upon closing a circuit impressing E.M.F. from a steady D.C. source. String tension as in figure 16 C. Interval between vertical time lines = 0.2 second.

Fig. 19. Same as figure 18. Time lines = 0.01 second.


7
8
9
10
11
12
14
16
13
15
18
19
A
B
C
D.C.
A.C.
1000 Ω/VOLT
TO AMPLIFIER
MM. PER MICROVOLT
CYCLES PER SEC.
17

Figs. 6–19

of distortion, both as to magnitude and phase, results from lag of the string (e.g., see Fulton, 1925a). 4. The present amplifier, as here used, was somewhat underdamped, resulting in an oscillatory discharge when E.M.F. from a steady D.C. source is suddenly impressed by closing a circuit (figs. 18 and 19).

It is believed that these difficulties can be overcome eventually, but that the present results are accurate within the frequency limits stated, and that this is sufficient for purposes of the present investigations.

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THE LONGEVITY OF THE ERYTHROCYTE

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At least three different methods have been employed by investigators in seeking to estimate the rate of overturn of the blood in mammals. The first and probably the most direct method involves an attempt at a determination of the duration of "life" of the erythrocyte in the circulation. It is with this approach that the present paper is concerned.

The second method is one employing the output of a bile pigment, bilirubin, a derivative of hemoglobin, as an index of erythrocyte destruction. By this technique Goodman (1907) reported that about 2 per cent of all the circulating erythrocytes are destroyed every day. Zoja (1910) estimated that 10 per cent of the red cells are renewed daily and Brugsch and Retzlaff (1912) reached the same conclusions but Eppinger and Charnas (1913) criticized their technique and reported that 3 per cent of the total erythroplastid volume is removed per day.

The third approach to the study of the blood overturn has been made through the measurement of the time requisite for replacement regeneration to occur after removal of a known fractional part of the hemoglobin or of the corpuscular volume. From hemoglobin regeneration rates Whipple and Robscheit (1925) estimated that 11.5 grams of hemoglobin constitute the portion destroyed per week as normal functional "wear and tear" (maintenance factor) in dogs having a blood volume of approximately 550 cc. They indicate the blood overturn requires about five weeks. The inadequacies of the three methods will be considered in the final discussion.

In this paper an original technique is described for the determination of the duration of life of the circulating erythrocyte. In the early studies on erythrocyte longevity such as those of Ward-Muller and of Von Ott (quoted by Ashby, 1919) the red corpuscles from one animal were transfused into another and the time for their disappearance from the recipient's circulation noted. The most careful work, however, was that of Ashby (1919, 1921) using the transfusion method but applying the modification of identifying the transfused corpuscles by the agglutination technique of compatible blood groups. She found that it took from 30 to 100 days for the injected corpuscles to disappear from the host circulation in man.

Other workers have criticized these figures as too high and not representative of the life duration of the corpuscle when in its own circulation, for the transfused erythrocytes are apparently treated as foreign bodies (Wearn et al., 1922; Todd and White, 1911; Rous, 1923) and hence may survive for long periods. A more significant objection lies in the fact that the cells taken from the donor's blood are already in all stages of senescence and hence complicate interpretations of their disappearance time. Furthermore, Görl (1926) using Ashby's technique finds variations are too great to render it valid in determining the longevity of the erythrocyte. Wearn, Warren, and Ames (1922) and others using Ashby's technique report results similar to Ashby's. Schulten's (1930) technique is however more satisfactory.

Eaton and Damren (1930) have described a method which does not require transfusion and depends on reticulocyte response after artificial hemorrhage. The hemorrhage induces a series of successive peaks in the reticulocyte-count curve. These peaks are separated by time intervals equivalent to the life duration of the erythrocyte, for the second peak represents a second reticulocyte response due to the simultaneous mass death of the cells that had entered the circulation as reticulocytes following the hemorrhage and which have lived out their life span. They thus report the life duration of the rabbit's erythrocyte as being 8 days.

MATERIALS AND METHODS. I. *Experiments on rats.* We have devised another method for estimating the longevity of the erythrocyte directly without recourse either to transfusion or to reticulocyte counts. We increase the number of erythrocytes in the blood by exposing the animal to low oxygen pressures for a short period of time (2 to 14 days). The animal is then exposed to normal atmospheric pressure and blood counts are taken daily to determine the time interval required for the normal red-cell count and red-cell volume to be attained. The most extensive series of our experiments consisted of an examination of 30 albino rats. For each rat its own characteristic erythrocyte count (normal) was carefully predetermined. In this manner the return to the normal for each rat was more accurately detectable. After the removal of each animal from the respiration chamber blood counts were made every other day as indicated. In every case 300 squares in the counting chamber (Neubaur ruling) were inspected. Only two drops of blood were drawn from the tail tip, enough blood for dilution in three pipettes. One pipette was of the Bureau of Standards calibration, the others were of the standard calibration in common clinical use. Hayem's dilution fluid was used.

In view of the rat's limited blood volume which is susceptible to considerable reductions upon the withdrawal of even the small amounts required for hematocrit or plasma-dye readings, it was considered wiser to determine, before the blood-count experiments were undertaken, whether a

concentration or "expansion" of the plasma occurred with confinement to the low oxygen for periods of time equivalent to those to be repeated later for the cell count determinations. Each of four rats was subjected to 2, 4, 8, and 12 day exposures to the low pressure of oxygen (90 mm. Hg) and determinations of each rat's plasma volume were made by the dye and colorimeter method described by Hooper (1920). Values for plasma volume for each rat before exposure and at the end of the exposure period were not found to vary more than within the limits of error for the technique employed. Volumes of plasma and variations in each rat were 7 ± 0.1 cc., 7.2 ± 0.05 cc., 6.9 ± 0.04 cc., 7.1 ± 0.07 cc. There is ample evidence in the literature that increases in erythrocytes do not entail changes in plasma volumes in cases of exposures to low oxygen tensions (Smith, Belt, Arnold, and Carrier, 1924, and Campbell, 1927).

The respiration chamber was prepared from a triple-walled constant-temperature incubator by rendering it completely air-tight after painting and sealing the interior and exterior with a specially prepared rubber-paraffine vacuum-seal wax. Double doors, heavily padded with felt which had previously been permeated with the vaseline, blocked the ingress of air. A mercury-seal valve was used for regulating the partial pressure of oxygen in the chamber. The oxygen pressure was maintained throughout every experiment at 90 ± 5 mm. of mercury. For rapid lowerings of the oxygen pressure such as are required after opening the door of the chamber for introducing the animal or its drinking water, a hydraulic vacuum pump was used. After the desired oxygen pressure (90 mm.) is attained, it is held almost constant by permitting a slight ingress of air through the mercury valve from time to time,—this compensates for the animals' respiratory requirements as revealed by manometer readings. The carbon-dioxide expired is removed from the air by an adequate commercial mixture of calcium-chloride and sodium-hydroxide. Campbell (1927) has found respiration chambers and accessories, such as we have used, accurate and satisfactory for studies in low oxygen respiration. The oxygen and carbon-dioxide concentrations in the chamber are easily determinable by the methods of Henderson (1918), of Binger (1925), and of Higgins and Marriott (1917). Only for one minute every other day were the animals exposed to normal pressure while water was given. No food was given the animals during the period of confinement in the chamber. The urine was absorbed by layers of sawdust and blotting papers arranged below the animals' platform. All rats used in these experiments were adult males weighing about 300 grams, ages 360 to 450 days; 21 rats were divided into groups of three each. Each group was exposed to the low oxygen tension (90 mm.) for periods of from 2 days to 14 days as is indicated at the heads of the columns in table 1. The temperature was maintained at 32 to 33°C. throughout.

Table 1 shows that in the two and four day exposures at whose termination the red cells had increased only from 9 to 11 million per cu. mm. the erythrocytes in these cases return to normal in from 11 to 13 days, whereas in longer exposure experiments on whose termination the red cells numbered 12 to 16 million per cu. mm. the attainment of the normal count took place after 12 to 15 days. It is evident also from the table that only in the long exposure periods is there a very rapid decrease in the number of erythrocytes during the first two or three days of respiring atmospheric oxygen, amounting to as much as 3 million per cu. mm. in the high-count cases. After this rapid drop the rate of decrease is more gradual, reaching normal at about the 12th day and continuing to drop to counts as low as

TABLE 1

Experiments on rats

DAYS OUT	14 DAYS' EXPOSURE			14 DAYS' EXPOSURE			9 DAYS' EXPOSURE			8 DAYS' EXPOSURE			6 DAYS' EXPOSURE			4 DAYS' EXPOSURE			2 DAYS' EXPOSURE		
	A9	A5	A2	A8	G7	G6	B2	B3	B5	C1	C4	C5	D1	D2	D3	M7	M8	M9	N4	N6	N7
1	16.6	16.6	15.7	15.8	15.2	15.7	15.2	15.3	15.0	14.5	14.8	14.3	12.8	13.1	12.5	11.1	10.9	11.3	9.9	9.7	9.8
2																					
3	13.7	11.5	12.8	12.7	12.5	12.6	12.5	12.2	12.3	13.2	13.3	13.2	12.1	12.3	12.0	10.8	10.4	11.0	9.4	9.5	9.5
4																					
5	11.8	10.5	11.3	11.5	11.6	11.3	11.3	11.1	11.0	12.1	12.0	12.2	11.5	11.7	11.6	10.4	10.0	10.8	9.4	9.4	9.4
6																					
7	11.1	9.8	10.8	11.0	10.9	10.8	10.6	10.4	10.4	11.7	11.7	11.6	11.0	11.2	11.4	10.2	9.7	10.6	9.2	9.3	9.4
8																					
9	10.5	8.5	10.3	10.5	10.3	10.4	10.0	9.9	9.8	11.2	11.3	11.0	10.7	10.8	10.9	9.8	9.4	10.3	9.0	9.1	9.2
10																					
11	9.9	**7.3**	9.7	9.9	9.8	9.9	9.6	9.5	9.4	10.5	10.6	10.5	10.2	10.4	10.5	9.5	9.1	10.2	**8.8**	**9.0**	**8.9**
12																					
13	9.2	5.7	9.4	9.6	9.4	9.5	9.3	**9.2**	**9.1**	9.9	9.9	9.8	9.8	9.9	9.9	**9.3**	**8.9**	**10.1**	8.7	8.8	8.6
14																					
15	**8.7**	5.5	**9.2**	**9.4**	**9.0**	**9.1**	**9.1**			**9.4**	**9.3**	**9.4**	**9.5**	**9.0**	**9.4**						

The numbers in the columns indicate the decreasing red-cell counts in millions per cubic millimeter.

At the left is indicated the number of days after removal of the rat from the low oxygen.

At the heads of the columns are indicated the exposure times to low oxygen pressure for each series, with the numbers of individual rats.

The numbers in bold type indicate the normal count and the time of its attainment.

5.5 million. Since in the cases where the total erythrocyte increase amounted to only 2 or 3 million above normal, the sudden massive decrease of the first 3 atmospheric-respiration days did not occur, as in the high count cases, we are of the opinion that the rate of red cell destruction (and also longevity) is proportional to the erythrocyte population density in the circulation. During the three post-exposure days the drop in the number of red cells occurs as a compensation to reduce the doubled cell volume of the blood, but still leaves enough super-abundant cells to enable one to recognize with certainty the day on which the normal count is reached, after a time interval representing the normal life span of the red corpuscle.

Campbell's (1927) studies indicate that in short periods of exposure to low oxygen hemopoietic hyperactivity ceases almost at once on removal of the animal from the subnormal oxygen. Since during the period of low oxygen respiration the hyperhemopoiesis and hyperdestruction attain an equilibrium, little further increase in red cell count occurs after a certain period. However, on the sudden cessation of high marrow activity the cell-destroying mechanism is left to lag behind for only 2 or 3 days. Hence, the resultant anemia appearing after about 15 days is more probably due to a subnormal activity of the marrow than to hyperactive cell destruction.

Table 1 shows that in all the short-exposure cases the normal count is attained on the 11th to the 13th day after removal of the rat from the chamber. Whether this represents the normal longevity of the erythrocyte or whether to this value must be added one-half of the number of days constituting the period of low oxygen exposure is the point in question. The latter procedure seems the more logical provided that the exposure period be not more than 7 days, for exposures of longer duration to low tensions of oxygen hyperactivate the marrow and supernormal erythrocyte formation continues for long periods after removal of the animal from the respiration chamber. From our technique we suggest that the longevity of the rat's erythrocyte lies between 12 and 18 days. Cartland and Koch (1928) report that rats regenerate their blood "completely every 10 to 13 days." Also Myers, Beard, and Barnes (1932) report "about 2 weeks" for increased erythrocyte counts to reach normal. Hart, Steenbock, Elvehjem and Waddel (1925) report 15 days for a definite drop in red cells to occur in experimental anemias. On the other hand, Jencks (1922) reports complete regeneration of the blood in rats in 18 days, and Geiling and Green (1921) also report 18 days.

II. *Experiments on dogs.* Employing the same technique as previously described for the rats, we have obtained similar data for dogs. In the latter case, however, the respiration chamber was a small refrigerator room rendered airtight by tarring and greasing the interior. The oxygen pressure maintained throughout the exposures was 90 mm. Hg as with the rats. This was regulated as before by use of the vacuum pump, mercury-seal valve, and attached manometer. All analyses and precautions were taken as previously described for the rats. No significant plasma volume changes were noted. Since only three dogs were used no complete protocol is given. In dog 5 the erythrocytes rose from 7.2 to 9.0 millions per cu. mm. in 7 days, and returned to normal in 16 days. In dog 12 the rise in red cells was from 7.3 to 9.5 millions in 8 days and back to 7.3 in 14 days. In dog 14 erythrocytes increased from 7 to 9.3 million in 7 days and the return to normal took 15.5 days. From this and other evidence it appears that the duration of life of the dog's erythrocyte is about 18 ± 3 days. Eaton and Damren (1930), analyzing Isaac's data, assign about 16 days'

longevity to the red blood cell of the dog. Our recalculations from the data of Camero and Krumbhaar (1933) indicate a longevity of 16 to 22 days for the erythrocyte in the circulation of the dog. On applying Eaton and Damren's analysis to data reported by Krumbhaar and Chanutin (1922) the longevity of the dog's erythroplastid falls around 15 days. Finally, Whipple (1922a) presents data on the regeneration of the dog's blood which suggests an erythrocyte longevity or an overturn of the blood of approximately 16 or 17 days.

Eaton and Damren (1930) in their two papers report a longevity of 8 or 9 days for the red corpuscle of the rabbit. Steele (1933) published data on reticulocyte responses in rabbits which by Eaton's hypothesis indicate that the rabbit's erythrocyte circulates for about 10 days. Again, data from Muller (1927), upon analysis, reveal a probable longevity for the rabbit's erythrocyte of from 7 to 10 days. These estimations receive further corroboration upon our recalculation of values reported by Boycott and Douglas (1910), by Ponder and Millar (1928), and by Robertson (1917).

III. *Experiments on man.* A series of experiments has been started to estimate the longevity of the erythroplastid in man, making use of the increase in the erythrocyte count due to short sojourns at high altitudes. The sites of residence were on Mount Baldy and on the Lake Arrowhead Mountains in southern California, near Los Angeles and San Bernardino, respectively. We can make a preliminary report of 5 cases observed to the present. Five men, ages 22 to 36, residing 14 to 16 days at altidudes of 9000 and 10,500 feet, showed increases after two weeks of residence from 5.3 and 5.2 to 6.0 and 5.9 millions per cu. mm. (2 individuals 70 and 61 kgm., respectively) and 3 other individuals (51, 68, 71 kgm.) showed, after 14 days' residence, counts of 6.3, 6.5 and 5.9 millions per cu. mm. In the first two persons (at 9000 feet) the red cell count, therefore, increased less than 1 million per cu. mm. in two weeks, and after their descent to sea level which required one day, the blood-cell count dropped to normal on the 20th day. In the other three individuals sojourning for 2 weeks at 10,500 feet, their erythrocyte increase of about 1.5 million per cu. mm. required 18, 21, and 20 days respectively to return to the normal of 5.2 million. The validity of this method in estimating the longevity of the red blood cell depends primarily on the length of exposure to the low oxygen of the altitudes and upon the extent of increase in the erythrocyte count. The method then is valid if the residence period at the altitudes is not longer than two weeks and provided that the increase in the red cells does not exceed 2 million per cu. mm. If the cell-count is too great the compensatory erythro-destroying mechanism begins to act and the supernumerary cells under observation may not live out their allotted normal life-span. Again, if the period spent at the heights is long, the addition

of one-half of its duration in days to the period requisite for a return of the cell count to normal (after the subject's descent) magnifies and distorts the actual life-duration of the erythrocyte. Since Smith, Belt, Arnold and Carrier (1924) have shown that a definite increase in the erythrocyte volume is first detectable at 2 weeks of residence at altitudes of over 10,000 feet, we have considered a two-week sojourn the minimum period required for an increased erythrocyte count to be positively due to an absolute increase in the number of red cells. Yet, the two-week period at the heights is short enough to prevent the hyperhemopoiesis to be continued after descent, as happens in cases of sojourns of over two months. Smith, Belt, Arnold, and Carrier, who remained for one month at 11,000 feet, show that the erythrocyte volume and blood-counts returned to normal in from 16 to 24 days after descent. In our subjects during the period of cell decrease, erythrocyte counts are taken every other day. The life span then, of the erythroplastid in man appears to lie between 18 and 30 days. This estimation is corroborated by data published by Walters and Woodard (1933) who increased the erythrocytes by liver feeding, in normal subjects. They wrote: "In nearly every case the fall (to normal, in erythrocytes) occurs 12 or 13 days after the feeding has been discontinued." We would add 3 or 4 days to these reported values since Walters and Woodard fed their subjects liver daily for about 8 days. Many clinical data on reticulocyte responses exist which are susceptible to the analysis suggested by Eaton and Damren and probably indicate roughly the life span of the red blood cell. Data from Minot, Murphy, and Stetson (1928), from Riddle and Sturgis (1930), and from Bloomfield and Wyckoff (1929) show a probable longevity of from 16 to 21 days for the red blood corpuscle of man. As a suggestion, we may mention that throughout all of these experiments D. Tyler, studying leucocyte longevity with the authors has noticed a large white cell increase on exposure of the animals to low oxygen. This increase was also reported by Campbell (1927).

Discussion. Whipple and Hooper (1917), Whipple (1922b) and Rous (1923) have pointed out that the quantitative output of bilirubin can not be used as an index of erythrocyte destruction. Carbohydrate consumption considerably affects the amount of bilirubin secreted, indicating a complex intermediate metabolism. Again, muscle hemoglobin is also represented in bilirubin. Furthermore, if a basal output of bilirubin representative of both erythrocyte and muscle hemoglobin is estimated and the red cells increased in the animal by transfusion, for determining the bilirubin increase, the argument regarding the abnormal rate of destruction of foreign erythrocytes in a recipient's circulation comes into play. Finally, if free hemoglobin is injected into the circulation its rate of removal from the plasma (as bilirubin) will yield no indication of the rate of erythrocyte destruction.

The regeneration method whereby the replacement time for a known fractional part of the corpuscular blood is taken to indicate the proportional time requisite for a renewal of the whole cell volume, is also open to serious objections. Studies on erythrocyte regeneration show that the hemorrhage preceding regeneration constitutes a supernormal stimulus to the erythrogenic marrow, so that fractional parts of the blood after hemorrhage are undoubtedly replaced more rapidly than equivalent parts under normal "wear and tear." Again, the studies of Whipple and Robscheit (1925) and of Robscheit (1929) show that the rate of erythrocyte regeneration varies enormously with the diet so that it is almost impossible to estimate the time required for one normal overturn of the blood from data on the regeneration rate of fractional parts. Such factors probably account for the differences on the rate of blood overturn in rats obtained on the one hand, by Geiling and Green (1921), Jencks (1922) and, on the other, by Cartland and Koch (1928). Whipple and Robscheit's (1925) experiments on the determination of the "maintenance factor" of hemoglobin, which indicated a blood overturn of five weeks, are open to criticism. Whipple and Robscheit performed these experiments on dogs whose erythrocyte volume (or red cell count) was half that present in normal animals so that the frictional cell destroying mechanism, which we find to be greatly dependent on the population density of circulating cells, was undoubtedly operative to a much less extent than under normal conditions. If this is taken into account their overturn estimation agrees with ours.

The following points regarding our own technique are noteworthy: Since an individual group of cells is not followed as in the case of identification by agglutination, the return of the cell count to normal is due to the removal, not of the cells thrown into circulation during the period of low oxygen exposure but to the withdrawal of older erythroplastids which were present in the circulation before the exposure period. When the erythrocyte increase is less than double the normal cell volume the return of the high count to the normal is not characterized by a destruction of a mass of cells equivalent to the mass removed in the course of one normal blood overturn. Hence it may be argued that since the mass destroyed is less, the time required for the destruction may also be less than that of an overturn. But it is evident from the data that the time requisite for attainment of the normal erythrocyte volume and count is approximately the same whether the increase is only one million cells per cubic millimeter or eight million. Hence it is permissible to employ increases of only one to three million cells per cubic millimeter on account of certain disadvantages, previously mentioned, incurred when obtaining larger counts.

As already indicated we have noticed that in the cases where the erythrocyte volume approached double the normal value a very rapid destruction of red cells occurred early in the post-exposure period, but gradually

diminished as the density of the erythrocyte population decreased. The rate of decrease fell off so rapidly that the normal count was reached almost in the same time by large as by small increments of corpuscles. This indication that rate of red cell destruction is regulated by the density of the erythrocyte population has received corroboration from experiments left for a later report. The finding by Robertson and Rous (1917) that a general fragmentation of erythrocytes takes place throughout the circulatory channels certainly argues in favor of the existence of some such frictional mechanism in blood cell destruction.

The withdrawal of the small blood samples necessary for the blood counts, at two day intervals, was found to have no effect on the rate of return of the increased erythrocyte counts to normal (in controls).

Our experiments leave no doubt that a species difference in erythrocyte longevity exists. However, we are of the opinion that the density of the circulating erythroplastid population is as important a factor in determining the life duration of the corpuscle as is fragility. The following table shows the relation between red cell longevity and cell population density (erythrocyte count):

Rat—12 to 18 days (cell longevity)—*8 to 9* million red cells per cu. mm.
Dog—16 to 22 days (cell longevity)—*7 to 8* million red cells per cu. mm.
Man—23 to 30 days (cell longevity)—*4.5 to 5.5* million red cells per cu. mm.

It is very possible that the longevity of the erythroplastid may be inversely proportional to the population density of erythrocytes or to some relationship between population density and cell fragility.

SUMMARY

1. The longevity of the erythrocyte in mammals may be determined by increasing the erythrocyte volume in the circulation by means of short exposure periods to low pressures of oxygen. The number of days elapsing from the end of the exposure period to the attainment of the normal red cell volume (or count) indicates approximately the duration of life of the erythroplastid in the circulation.

2. The longevities of the erythrocytes in the following species were found to be: 1, rat, 12 to 18 days; 2, dog, 16 to 23 days, and 3, man, 18 to 30 days.

3. The longevity of the erythrocyte or the period of one overturn of the blood is apparently regulated by some relationship between the population density of circulating erythroplastids and the fragility of the red cells.

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